Oligosaccharide Esters from the Roots of *Polygala arillata*

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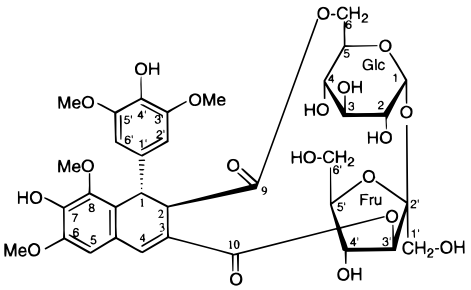
Two new sucrose esters, arillatoses A (**1**) and B (**2**), and four new trisaccharide esters, arillatoses C−F (**3**−**6**), were isolated from the roots of *Polygala arillata*, together with four known sucrose esters, glomeratose E (**7**) and sibiricoses A_1 (**8**), A_5 (**9**), and A_6 (**10**). The structures of the new compounds were elucidated on the basis of chemical and spectroscopic evidence.

In the course of conducting a research program on the oligosaccharide esters from *Polygala* species,[2] we have investigated *P. arillata* Buch.−Ham. (Polygalaceae). This species is widely distributed in the People's Republic of China, and its roots are used as a traditional medicine in a manner similar to "Yuan zhi" (the roots of *P. tenuifolia* Willd.) to tranquilize, as a tonic, and to prevent loss of memory.[3] No previous investigation has been reported on the oligosaccharide esters of *P. arillata*. We now report the isolation and structure elucidation of two sucrose esters, arillatoses A (**1**) and B (**2**), and four new trisaccharide esters, arillatoses C−F (**3**−**6**). Four known compounds isolated from this plant were identified by comparison of the spectral data with reported data as glomeratose E (**7**)[1] sibiricoses A_1 (**8**), A_5 (**9**), and A_6 (**10**).[2]



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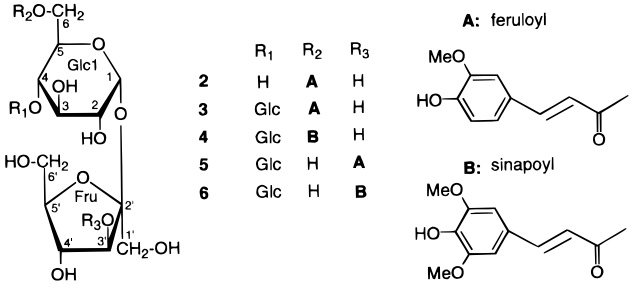

Results and Discussion

The air-dried roots of *P. arillata* were extracted with MeOH under reflux. The MeOH extract was suspended in H_2O and extracted with ether. The H_2O layer was adsorbed on a porous polymer gel (Diaion HP-20) column and eluted with mixtures of water and methanol. The 50% MeOH eluate was chromatographed further to afford six sucrose esters (**1**, **2**, and **7**-**10**) and four trisaccharide esters (**3**−**6**).

The FABMS of arillatose A (**1**) showed quasimolecular ion peaks at *m*/*z* 753 $[M + H]^+$ and 775 $[M + Na]^+$, consistent with a molecular formula of $C_{34}H_{40}O_{19}$. On alkaline hydrolysis, **1** afforded sucrose, while on acid hydrolysis, it gave D-glucose and D-fructose.[4] The ^{1}H NMR spectrum of **1** exhibited two methine protons [δ 4.81 (1H, br s) and δ 3.98 (1H, d, J = 2 Hz)], a vinyl proton at a highly deshielded position [δ 7.77 (1H, s)], and two aromatic protons [δ 6.92 (1H, s) and δ 6.34 (2H, s)], in addition to the signals due to sucrose. The aromatic proton (δ 6.34) indicated the presence of a 1,3,4,5-tetrasubstituted benzene unit in **1**. All proton and carbon signals were assigned by COSY, HOHAHA, HMBC, and HMQC NMR experiments. On irradiation of the aromatic proton at δ 6.34 due to H-2′, ROEs were observed at a methoxyl at δ 3.70 (6H, s), a methine proton at δ 4.81 due to H-1, a methine proton at δ 3.98 due to H-2, and an olefinic proton at δ 7.77 due to H-4 (Figure 1). From this ROE correlation between H-2′ and H-4, the bond C-1−C-1′ was concluded to be quasi-axial. The coupling constant between H-1 and H-2 (4 Hz) indicated that the bond C-2−H-2 was quasi-equatorial, and if the bond C-2−H-2 had been quasi-axial, the H-4 signal would have been a doublet induced by the allylic coupling with H-2.[5] The CD spectrum of **1**, which is identical with (1*S*,2*R*)-1,2-dihydro-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)-naphthalene 2,3-dicarboxylic acid dimethyl ester,[6] showed a positive first Cotton effect at 346 nm. Therefore, **1** has a 1*S*,2*R* configuration. From these data, the structure of arillatose A was deduced as cyclic 3′→3:6→2-[(1*S*,2*R*)-1-(4-hydroxy-3,5-dimethoxyphenyl)-1,2-dihydro-7-hydroxy-6,8-dimethoxy-2,3-naphthalenedicarboxyl]-β-D-fructofuranosyl α-D-glucopyranoside.

Arillatose B (**2**) was isolated as an amorphous powder. The positive mode FABMS revealed a quasimolecular ion peak at *m*/*z* 541 $[M + Na]^+$, consistent with a molecular formula of $C_{22}H_{30}O_{14}$. On alkaline hydrolysis, **2** afforded sucrose and ferulic acid, while on acid hydrolysis, it gave D-glucose and D-fructose. In the ^{1}H NMR spectrum of **2**, one feruloyl signal was observed, in addition to the signals due to sucrose. All proton and carbon signals in the NMR spectra (Tables 2 and 3) of **2** were assigned from its ^{1}H−^{1}H COSY, HOHAHA, HMBC, and HMQC spectra. The position of the feruloyl group in the sucrose moiety of **2** was deduced from the HMBC experiment. In this spectrum, a long-range correlation ($^3J_{COCH}$) was observed between the feruloyl carbonyl carbon signal at δ 169.2 and the proton

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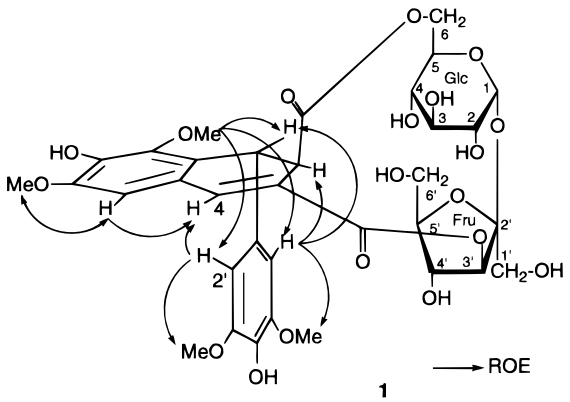

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Figure 1. Correlations observed in the ROE difference NMR spectrum of arillatose A (**1**).

Table 1. ^{1}H NMR and ^{13}C NMR Data of **1** in CD_3OD at 35 °C

		^{1}H NMR	^{13}C NMR	HMBC (C→H)
sugar moiety				
	Glc-1	5.53 d (4)	92.8	Fru-2
	2	3.37 dd (4, 10)	73.7	
	3	3.46 dd (10, 9)	75.6	Glc-2
	4	3.10 dd (10, 9)	72.1	Glc-3, −5
	5	3.70 m	73.2	Glc-6
	6	4.06 dd (10, 2)	65.7	α
		4.57 dd (10, 9)		Glc-5, α
	Fru-1	3.66 d (12)	66.7	
		3.77 d (12)		
	2		105.3	
	3	5.28 d (9)	80.3	Fru-4, α′
	4	4.33 dd (10, 9)	72.2	Fru-3, −5, −6
	5	3.66 [a]	81.9	Fru-2, −3
	6	3.76 [a]	61.0	
		3.86 [a]		
lignan moiety				
	1	4.81 br s	42.0	2, 8, 8a, 9, 1′, 2′, 3′,6′
	2	3.98 d (2)	49.6	1, 4, 9, 10, 1′
	3		123.1	
	4	7.77 s	141.5	2, 3, 4a, 5, 10
	4a		124.3	
	5	6.92 s	109.7	4, 4a, 6, 7
	6		149.5	
	7		143.8	
	8		146.9	
	8a		124.8	
	9		174.7	
	10		168.6	
	1′		135.4	
	2′	6.34 s	106.0	1, 1′, 3′, 6′
	3′		149.1	
	4′		135.6	
	5′		149.1	
	6′	6.34 s	106.0	1, 1′, 2′, 5′
	MeO-6	3.91 s	56.9	6
	MeO-8	3.53 s	60.8	8
	MeO-3′	3.70 s	56.8	3′
	MeO-5′	3.70 s	56.8	5′

[a] Overlapped.

signal at δ 4.27 due to H-6 of glucose. From these data, the structure of **2** was elucidated as β-D-fructofuranosyl 6-*O*-feruloyl-α-D-glucopyranoside.

Arillatose C (**3**) was obtained as an amorphous powder. The FABMS of **3** showed a quasimolecular ion peak at *m*/*z* 703 $[M + Na]^+$. Compound **3** gave D-glucose and D-fructose in the ratio 2:1 on acid hydrolysis, while on alkaline hydrolysis it afforded ferulic acid. In the ^{1}H NMR spectrum of **3**, one feruloyl signal was observed. Full assignments of the proton and carbon signals were secured by a HOHAHA difference spectrum, on irradiating at the glucosyl anomeric proton signal and H-3 of the fructosyl moiety, and from ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. In the HMBC spectrum, H-6 of Glc 1 was correlated to an ester carbonyl carbon at δ 169.1. In a ROE difference spectrum, ROE was observed at δ 3.92 (1H, dd, J = 9, 9 Hz) due to H-3 of Glc 1 on irradiation at the anomeric proton signal of Glc 2 at δ 4.53 (1H, d, J = 8 Hz). Accordingly, **3** was deduced as a *O*-β-D-glucopyranosyl-(1→3)-6-*O*-feruloyl-α-D-glucopyranosyl β-D-fructofuranoside.

The FABMS of arillatose D (**4**) showed a quasimolecular ion peak at *m*/*z* 733 $[M + Na]^+$. The ^{1}H NMR spectrum was similar to that of arillatose C (**3**), but it showed the presence of a sinapoyl residue. Compound **4** gave D-glucose and D-fructose in the ratio 2:1 on acid hydrolysis, while alkaline hydrolysis gave sinapic acid. The HMBC experiment showed a correlation between H-6 of Glc 1 and an ester carbonyl carbon at δ 169.0. Thus, **4** was determined as a *O*-β-D-glucopyranosyl-(1→3)-6-*O*-sinapoyl-α-D-glucopyranosyl β-D-fructofuranoside.

The ^{1}H NMR spectra of arillatoses E (**5**) and F (**6**) displayed patterns similar to those of arillatoses C (**3**) and D (**4**), respectively, except for downfield-shifted oxymethine protons due to H-3 of a fructosyl moiety at δ 5.44 (1H, d, J = 7.5 Hz). On alkaline hydrolysis, **5** afforded ferulic acid and **6** afforded sinapic acid. Compounds **5** and **6** gave D-glucose and D-fructose in the ratio 2:1 on acid hydrolysis. In the HMBC spectra of **5** and **6**, H-3 of a fructosyl moiety was correlated to an ester carbonyl carbon. Therefore, **5** was deduced as *O*-β-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl 3′-*O*-feruloyl-β-D-fructofuranoside and **6** as *O*-β-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl 3′-*O*-sinapoyl-β-D-fructofuranoside.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on Hitachi U-3410 spectrometer and CD spectra on a JASCO J-20A spectropolarimeter.^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL α-400 FT-NMR spectrometer with TMS as an internal standard. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulse-field gradient. Positive-mode FABMS were recorded on a JEOL JMS-SX102 spectrometer, using a *m*-nitrobenzyl alcohol matrix. GC was carried out with Hitachi G-3000 gas chromatograph. HPLC was performed using a JASCO System 800.

Plant Material. *P. arillata* Buch.-Ham. was collected in June 1996, in Sichuan, People's Republic of China. The plant was identified by Prof. Zhaoguang Liu, Chengdu Institute of Biology, Academia Sinica, People's Republic of China, and a voucher specimen (no. 960715) has been deposited in the Herbarium, School of Pharmaceutical Sciences, University of Shizuoka.

Extraction and Isolation. The dried and powdered roots of *P. arillata* (1.98 kg) were extracted twice with MeOH under reflux. After evaporation of the solvent under reduced pressure, the MeOH extract was suspended in H_2O and extracted with diethyl ether. The H_2O layer was subjected to passage over a porous polymer gel Mitsubishi Diaion HP-20 column (15 × 31.5 cm). The adsorbed material was eluted with 50% aqueous MeOH, 70% aqueous MeOH, and MeOH, successively, after washing with H_2O. The 50% aqueous MeOH eluate (11.7 g) was chromatographed on a Si gel (330 g) column using $CHCl_3$−MeOH−H_2O (80:18:2) as an eluent to afford fractions A−R. Fractions H + I (686 mg) were subjected to preparative HPLC [ODS 5 × 100 cm; CH_3CN−H_2O (9:91) → (17:83) linear gradient] to afford **2** (6 mg). Fractions J + K (565 mg) were subjected to preparative HPLC [ODS 5 × 100 cm; CH_3CN−H_2O (9:91) → (17:83) linear gradient] to afford **10** (86 mg). Fraction L (764 mg) was subjected to preparative HPLC [ODS 5 × 100 cm; CH_3CN−H_2O (8:92) → (16:84) linear gradient] to afford **1** (15 mg), **7** (31 mg), and **8** (16 mg). Fraction M (807 mg) was subjected to preparative HPLC [ODS 5 × 100 cm;

Table 2. ^{1}H NMR Data of **2**−**6** in CD_3OD at 35 °C

		2	3	4	5	6
Sugar moiety						
	Glc1−1	5.42 d (4)	5.47 d (3.5)	5.47 d (3.5)	5.48 d (4)	5.48 d (3.5)
	2	3.46 dd (10, 4)	3.68 dd (9, 3.5)	3.68 dd (9, 3.5)	3.62 dd (10, 4)	3.62 dd (10, 3.5)
	3	3.75 dd (10, 9)	3.92 dd (9, 9)	3.92 dd (9, 9)	3.65 dd (10, 10)	3.63 dd (10, 10)
	4	3.33 dd (9, 9)	3.44 dd (9, 10)	3.44 dd (9, 10)	3.49 dd (10, 10)	3.49 dd (10, 10)
	5	4.11 m	4.17 m	4.18 m	3.94 m	3.95 m
	6	4.27 dd (12.5, 6)	4.28 dd (12, 6)	4.28 dd (12, 6)	3.78 dd (12, 6)	3.78 dd (12, 6)
		4.51 dd (12.5, 2)	4.53 dd (12, 1.5)	4.53 dd (12, 1.5)	3.87 dd (12, 1.5)	3.87 dd (12, 3)
	Glc2−1		4.53 d (8)	4.53 d (8)	4.28 d (7.5)	4.25 d (7)
	2		3.30[a]	3.30[a]	3.23[a]	3.20[a]
	3		3.40 dd (9, 9)	3.40 dd (9, 9)	3.26[a]	3.26[a]
	4		3.30[a]	3.30[a]	3.27[a]	3.22[a]
	5		3.34 m	3.34 m	3.00 m	2.92 m
	6		3.65 dd (12, 6)	3.64 dd (12, 6)	3.54 dd (12, 6)	3.52 dd (12, 6)
			3.89[a]	3.89[a]	3.64[a]	3.60[a]
	Fru-1	3.60 d (12.5)	3.62 d (12)	3.61 d (12)	3.61 d (12)	3.61 d (12)
		3.60 d (12.5)	3.64 d (12)	3.63 d (12)	3.68 d (12)	3.69 d (12)
	3	4.09 d (8)	4.08 d (8)	4.08 d (8)	5.44 d (7.5)	5.44 d (7.5)
	4	4.06 dd (8, 8)	4.08[a]	4.08[a]	4.38 dd (9, 7.5)	4.39 dd (9, 7.5)
	5	3.80[a]	3.80[a]	3.80[a]	3.96[a]	3.97[a]
	6	3.78[a]	3.80[a]	3.80[a]	3.81[a]	3.81[a]
		3.82[a]	3.80[a]	3.80[a]	3.81[a]	3.81[a]
Acid						
	β	6.42 d (16)	6.43 d (16)	6.45 d (16)	6.44 d (16)	6.47 d (16)
	γ	7.63 d (16)	7.63 d (16)	7.62 d (16)	7.72 d (16)	7.71 d (16)
	2	7.22 d (2)	7.23 d (2)	6.94 s	7.24 d (2)	6.96 s
	5	6.81 d (7.5)	6.81 d (9)		6.84 d (9)	
	6	7.09 dd (7.5, 2)	7.09 dd (9, 2)	6.94 s	7.14 dd (9, 2)	6.96 s
	OMe	3.89 s	3.89 s	3.88 s	3.91 s	3.90 s

[a] Overlapped.

Table 3. ^{13}C NMR Data of **2**−**6** in CD_3OD at 35 °C

		2	3	4	5	6
sugar moiety						
	Glc1−1	93.3	93.0	92.9	93.1	93.1
	2	73.2	72.3	72.3	72.1	72.1
	3	74.7	85.4	85.4	86.9	86.8
	4	71.9	70.3	70.4	69.8	69.9
	5	72.1	71.9	71.9	74.4	74.4
	6	65.1	65.0	65.2	62.4	62.4
	Glc2−1		105.3	105.2	105.3	105.4
	2		75.5	75.5	75.5	75.5
	3		77.9	77.9	77.6	77.6
	4		71.5	71.6	71.1	71.0
	5		78.2	78.2	77.9	77.8
	6		62.6	62.6	62.3	62.1
	Fru-1	64.3	64.4	64.5	65.5	65.5
	2	105.2	105.2	105.3	105.5	105.5
	3	79.4	79.5	79.5	80.0	80.0
	4	76.2	76.2	76.2	74.2	74.2
	5	83.9	84.0	84.0	84.6	84.7
	6	64.1	64.2	64.3	62.9	62.9
acid						
	α	169.2	169.1	169.0	168.3	168.2
	β	115.4	115.4	115.9	115.2	115.6
	γ	147.0	147.0	147.2	147.7	147.9
	1	127.8	127.8	126.7	127.7	126.6
	2	111.8	111.8	107.1	112.1	107.4
	3	149.4	149.4	149.5	149.5	149.5
	4	150.6	150.6	139.7	150.9	139.9
	5	116.5	116.5	149.5	116.7	149.5
	6	124.2	124.2	107.1	124.5	107.4
	OMe	56.6	56.6	57.0	56.6	57.1

CH_3CN-H_2O (8:92) → (16:84) linear gradient] to afford **2** (23 mg), **8** (16 mg), **9** (61 mg), and **10** (18 mg). Fraction N (748 mg) was subjected to a preparative HPLC [ODS 5 × 100 cm; CH_3CN-H_2O (8:92) → (16:84) linear gradient] to afford **3** (9 mg), **7** (53 mg), and **9** (15 mg). Fraction O (812 mg) was subjected to a preparative HPLC [ODS 5 × 100 cm; CH_3CN-H_2O (8:92) → (16:84) linear gradient] to afford **3** (19 mg), **4** (13 mg), **5** (33 mg), and **6** (23 mg).

Arillatose A (1): amorphous powder, $[\alpha]^{27}_D$ +25.1° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.69), 250 (4.34), 340 (4.17) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 346 (+7.4), 253 (−6.4), 229 (−12.9) nm; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m*/*z* 775 $[M + Na]^+$, 753 $[M + H]^+$.

Arillatose B (2): amorphous powder, $[\alpha]^{27}_D$ +15.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (3.73), 295 (3.66), 326 (3.76) nm; ^{1}H and ^{13}C NMR, see Tables 2 and 3; FABMS *m*/*z* 703 $[M + Na]^+$.

Arillatose C (3): amorphous powder, $[\alpha]^{27}_D$ +15.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.81), 295 (3.75), 326 (3.83) nm; ^{1}H and ^{13}C NMR, see Tables 2 and 3; FABMS *m*/*z* 703 $[M + Na]^+$.

Arillatose D (4): amorphous powder, $[\alpha]^{27}_D$ +2.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.21), 330 (4.13) nm;^{1}H and ^{13}C NMR, see Tables 2 and 3; FABMS *m*/*z* 733 $[M + Na]^+$.

Arillatose E (5): amorphous powder, $[\alpha]^{27}_D$ −20.6° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.44), 240 (4.05), 294 (3.94), 327 (4.01), 380 (3.35) nm; ^{1}H and ^{13}C NMR, see Tables 2 and 3; FABMS *m*/*z* 703 $[M + Na]^+$.

Arillatose F (6): amorphous powder, $[\alpha]^{27}_D$ −4.5° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.50), 240 (4.17), 330 (4.12) nm; ^{1}H and ^{13}C NMR, see Tables 2 and 3; FABMS *m*/*z* 733 $[M + Na]^+$.

Alkaline Hydrolysis of 1−**6**. Each compound (2 mg) was treated with 1 N NaOH aqueous (50 μL) for 4 h at room temperature in N_2 atmosphere, and the reaction mixture was extracted three times with EtOAc after acidification with 1 N HCl. From the H_2O layer, a sugar was detected by HPLC [Asahipak NH2P-50, 4.6 mm × 25 cm, CH_3CN-H_2O (65:35), 1.0 mL/min, UV 195 nm,[7]] as follows: sucrose (t_R 5.2 min) from **1** and **2**. From the EtOAc layer, ferulic acid (t_R 9.1 min) was detected from **2**, **3**, and **5**; sinapic acid (t_R 8.6 min) was detected from **4** and **6** by HPLC [YMC R−ODS-5, 4.6 mm × 25 cm, CH_3CN-H_2O (22.5:77.5) + 0.05% CF_3COOH, 1.0 mL/min, UV 270 nm].

Acid Hydrolysis of 1−**6**. Each compound (1 mg) was heated on a boiling water bath with 1 N HCl (50 μL) for 15 min. The reaction mixture was passed through an Amberlite IRA-60E column and the eluate was concentrated. The residue was warmed at 60 °C with a solution of D-cysteine methyl ester in pyridine (3 mg/25 μL) for 90 min and to the reaction mixture hexamethyldisilazane (10 μL) and trimethylsilyl chloride (10 μL) were added, and the reaction mixture was stirred at 60

°C for 30 min. The supernatant was subjected to GC. Conditions: column Supelco SPB-1, 0.25 mm × 27 m; temperature 220 °C; carrier gas, N_2. From **1**−**6**, D-glucose (t_R 18.6 min) and D-fructose (t_R 14.3 min) were detected.[8] In **3**−**6**, the ratio of these sugars was 2:1.

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(8) The t_R for L-glucose (17.1 min) and the t_R for L-fructose (15.0 min) were obtained from that of the enantiomer (D-fructose + L-cysteine methyl ester).

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