A New Ionone Glucoside and a New Phenylpropanoid Rhamnoside from Stems of *Ampelopsis brevipedunculata* (MAXIM.) TRAUTV.¹⁾

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A new ionone glucoside and a new phenylpropanoid rhamnoside, termed ampelopsisionoside (1) and ampelopsisrhamnoside (2), respectively, have been isolated from stems of *Ampelopsis brevipedunculata* (MAXIM.) TRAUTV. (Vitaceae) and their structures have been established based on lines of chemical and spectral evidence. Four known glycosides, tachioside (3), isotachioside (4), lyoniside (5), and 2-phenylethyl D-rutinoside (6), were also isolated.

Keywords *Ampelopsis brevipedunculata*; Vitaceae; stem; ionone glucoside; ampelopsisionoside; phenylpropanoid rhamnoside; ampelopsisrhamnoside

Leaves and roots of *Ampelopsis brevipedunculata* (MAXIM.) TRAUTV. (nobudou in Japanese) (Vitaceae) are used to prepare the Chinese crude drugs named She-pu-tao and She-pu-tao-gen, respectively, which are employed in China as remedies for nephritis and rheumatism, and as an external hemostatic for traumatic bleeding, etc.²⁾ So far, flavonol glycosides,^{3a,b)} and *p*-coumaric and caffeic acid derivatives,^{3b)} have been isolated from leaves of *A. brevipedunculata*, and oligostilbenes⁴⁾ from roots of *A. brevipedunculata* var. *hancei* REHDER. As part of our studies on the constituents of Vitaceae, we have now examined stems of *A. brevipedunculata*. This paper deals with the structure elucidation of a new ionone glucoside and a new phenylpropanoid rhamnoside, and identifications of four known glycosides.

After chromatographic and high-performance liquid chromatographic (HPLC) separation of the *n*-BuOH soluble part of the MeOH extract, two new glycosides, termed ampelopsisionoside (1) and ampelopsisrhamnoside (2), have been isolated together with four known glycosides, i.e., tachioside (3),⁵⁾ isotachioside (4),^{5,6)} lyoniside (5),⁷⁾ and 2-phenylethyl D-rutinoside (6).⁸⁾

Ampelopsisionoside (1), a white powder, $[\alpha]_D -35.1^\circ$ (MeOH) showed carbonyl absorption at 1720 cm^{-1} in the infrared (IR) spectrum and had the molecular formula $\text{C}_{19}\text{H}_{32}\text{O}_8$ based on the $[\text{M}-\text{H}]^-$ peak at m/z 387 in the

negative ion fast atom bombardment mass spectrum (FAB-MS). The electron impact mass spectrum (EI-MS) of 1 showed no molecular ion peak but gave two significant fragment ions at m/z 226 $[\text{M}^+ - 162$ (hexose unit)] and 208

TABLE I. ^1H -NMR (400 MHz) Data for 1^{a)}

	1 ^{b)}	1 ^{c)}
2 α -H	3.14, d, 13.3	2.87, d, 13.4
2 β -H	2.05, dd, 13.3, 2.0	1.81, dd, 13.4, 2.1
4 α -H	2.67, t, 13.3	2.44, t, 13.4
4 β -H	2.30, m ^{d)}	2.12, ddd, 13.4, 4.6, 2.1
5 β -H	2.26, m ^{d)}	2.27, ddq, 13.4, 4.6, 6.4
7-H	5.86, dd, 15.9, 0.9	5.72, dd, 15.9, 0.9
8-H	6.37, dd, 15.9, 6.4	5.90, dd, 15.9, 6.4
9-H	4.75, quint, ^{e)} 6.4	4.44, quint, ^{e)} 6.4
10-H ₃	1.41, d, 6.4	1.32, d, 6.4
11-H ₃	1.16, s	0.99, s
12-H ₃	0.96, s	0.93, s
13-H ₃	1.01, d, 6.4	0.90, d, 6.4
6 α -OH	5.62, s	—
1'-H	4.96, d, 7.6	4.35, d, 7.9
2'-H	4.04, dd, 8.7, 7.6	3.18, t, 7.9
3'-H	4.26, t, 8.7	3.35, t, 7.9
4'-H	4.20, t, 8.7	3.31, t, 7.9
5'-H	3.92, ddd, 8.7, 5.2, 2.4	3.23, ddd, 7.9, 5.3, 2.4
6'-H ₂	4.33, dd, 11.6, 5.2	3.65, dd, 11.9, 5.3
	4.52, dd, 11.6, 2.4	3.84, dd, 11.9, 2.4

a) Chemical shifts are in δ -values from internal tetramethylsilane (TMS) and are followed by multiplicities and coupling constant (Hz). b) In pyridine-*d*₅. c) In MeOH-*d*₄. d) Both multiplicities and coupling constant were obscure due to partial overlap. e) Observed with fine splitting due to the long-range coupling (0.9 Hz) with 7-H.

TABLE II. ^{13}C -NMR Data for 1 (100.5 MHz, Pyridine-*d*₅, δ_C , ppm, from TMS)^{a)}

1		1	
1-C	43.11 (s)	11-C	25.30 (q) ^{c)}
2-C	52.08 (t)	12-C	24.71 (q) ^{c)}
3-C	210.67 (s)	13-C	16.61 (q)
4-C	45.87 (t)	1'-C	102.82 (d)
5-C	36.86 (d)	2'-C	75.50 (d)
6-C	77.17 (s)	3'-C	78.70 (d)
7-C	133.41 (d) ^{b)}	4'-C	71.95 (d)
8-C	134.61 (d) ^{b)}	5'-C	78.39 (d)
9-C	76.31 (d)	6'-C	62.95 (t)
10-C	21.42 (q)		

a) Assignments and multiplicities (in parentheses) were made with the aid of INEPT experiments. b, c) Assignments may be interchanged in each column.

Chart 1

$[M^+ - 162 - H_2O]$.

The proton nuclear magnetic resonance (1H -NMR) spectrum of **1** (Table I, in pyridine- d_5) showed signals ascribed to two tertiary methyls [δ 0.96, and 1.16], two secondary methyls [δ 1.01 (d, $J=6.4$ Hz) and 1.41 (d, $J=6.4$ Hz)], two olefinic protons [δ 5.86 (dd, $J=15.9, 0.9$ Hz) and 6.37 (dd, $J=15.9, 6.4$ Hz)], a hydroxyl proton [δ 5.62 (s)], and an anomeric proton [δ 4.96 (d, $J=7.6$ Hz)]. On methanolysis, **1** afforded methylglucoside as the sugar moiety. The results suggest that **1** is an ionone monoglucoside. The detailed 1H - and carbon-13 nuclear magnetic resonance (^{13}C -NMR) assignments (Tables I and II, respectively) were made with the aid of 1H - 1H correlation spectroscopy (1H - 1H COSY), nuclear Overhauser effect correlation spectroscopy (NOESY), and insensitive nuclei enhanced by polarization transfer (INEPT) techniques and it was inferred that **1** is an ionone glucoside with the relative structure **1** or its antipode, except for the configuration of the 9-OH group on the side chain.

The circular dichroism (CD) behavior of **1** showed a positive Cotton effect ($[\theta]$ +112) at 295 nm (MeOH) and the absolute stereochemistry of **1** was shown to be 5*R* (and 6*S*).⁹⁾ The structure of **1** was finally established as follows. The β -D-glucopyranosyl¹⁰⁾ moiety in **1** was corroborated by 1H -NMR [the anomeric proton signal with a large coupling constant ($J=7.6$ Hz)] (Table I) and ^{13}C -NMR data (Table II).

TABLE III. 1H -NMR (400 MHz) Data for **2** (MeOH- d_4)^{a)}

2	
2-H	6.84, d, 2.1
5-H	7.00, d, 8.2
6-H	6.73, dd, 8.2, 2.1
7-H ₂	2.66, m
8-H ₂	1.89, m
9-H ₂	3.36, 3.65, each 1H, m
OCH ₃	3.84, s
1'-H ₂	3.74, 3.76, each 2H, each d, 5.0
3'-H ₂	
2'-H	4.16, quint, 5.0
1''-H	4.64, d, 1.5
2''-H	3.79, dd, 3.4, 1.5
3''-H	3.65, m
4''-H	3.36, t, 9.5
5''-H	3.57, dd, 9.5, 6.4
6''-H ₃	1.23, d, 6.4

a) Chemical shifts are in δ -values from TMS and are followed by multiplicities and coupling constant (Hz).

TABLE IV. ^{13}C -NMR Data for **2** (100.5 MHz, MeOH- d_4 , δ_C , ppm, from TMS)^{a)}

2		2	
1-C	138.05 (s)	1'-C	62.16 (t)
2-C	114.21 (d)	2'-C	83.41 (d)
3-C	152.09 (s)	3'-C	62.16 (t)
4-C	152.09 (s)	1''-C	101.82 (d)
5-C	119.61 (d)	2''-C	72.59 (d) ^{c)}
6-C	122.01 (d)	3''-C	72.43 (d) ^{c)}
7-C	32.47 (t) ^{b)}	4''-C	74.09 (d)
8-C	33.08 (t) ^{b)}	5''-C	69.85 (d)
9-C	67.79 (t)	6''-C	18.03 (q)
OCH ₃	56.59 (q)		

a) Assignments and multiplicities (in parentheses) were made with the aid of INEPT experiments. b, c) Assignments may be interchanged in each column.

The position where the glucosyl residue is connected was made clear on the basis of the following NOESY experiments. A significant NOE cross peak between the anomeric proton of glucose (1'-H) and 9-H of the aglycone part was observed. This finding suggests that glucose is connected to the hydroxyl group at 9-C through a glycosidic linkage.¹¹⁾ Based on the combined evidence, the structure of ampelopsinonide is now established as **1**.¹⁴⁾

Ampelopsinonide (**2**), colorless oil, $[\alpha]_D -18.1^\circ$ (MeOH) had the molecular formula $C_{19}H_{30}O_9$ from high-resolution mass spectroscopic (HRMS) data (see Experimental). In addition, **2** gave two significant fragment ions at m/z 328 [$M^+ - 74$ (glycerol unit)] and m/z 255 [$M^+ - H - 146$ (deoxyhexose unit)].

The 1H -NMR spectrum of **2** (Table III) showed signals due to three aromatic protons [δ 6.73 (dd, $J=8.2, 2.1$ Hz), 6.84 (d, $J=2.1$ Hz) and 7.00 (d, $J=8.2$ Hz)], one methoxy group [δ 3.84 (s)], an anomeric proton [δ 4.64 (d, $J=1.5$ Hz)], and a secondary methyl [δ 1.23 (d, $J=6.4$ Hz)]. The detailed 1H - and ^{13}C -NMR assignments (Tables III and IV, respectively) were made with the aid of 1H - 1H COSY, NOESY, and INEPT techniques and it was suggested that **2** consists of three groups, i.e., a glycerol part [1H -NMR δ 3.74, 3.76 (2H each, each d, $J=5.0$ Hz) (1'-H₂ and 3'-H₂) or (3'-H₂ and 1'-H₂), 4.16 (quint, $J=5.0$ Hz) (2'-H); ^{13}C -NMR δ 62.16 (1'-C and 3'-C), 83.41 (2'-C)], a phenylpropanoid part, and an α -L-rhamnopyranosyl residue.¹⁵⁾ The connections between these three groups were determined as follows. In the NOESY experiments on **2**, significant NOE cross peaks between 2'-H of the glycerol and 5-H of the phenylpropanoid unit, and between the anomeric proton of rhamnose and 9-H₂ of the phenylpropanoid unit were respectively observed. This finding indicates that glycerol and rhamnose are connected to the hydroxyl group at 4-C and the hydroxyl group at 9-C of the phenylpropanoid unit through ether and glycosidic linkages, respectively. Further, the 1H - and ^{13}C -NMR data of **2** were analogous to those of compound **7**.¹⁶⁾ It was concluded that the 1-(3-methoxy-4-hydroxyphenyl)-glycerol unit in **7** was replaced by the glycerol unit in **2**. Based on the combined evidence, the structure of ampelopsinonide is established as **2**.¹⁷⁾

Experimental

The instruments used to obtain melting points, optical rotations, IR, 1H -NMR (400 MHz), ^{13}C -NMR (100.5 MHz), mass spectrum (MS), and gas liquid chromatography (GLC) data were the same as described in our preceding paper.¹⁹⁾ The CD spectrum was measured with a JASCO J-500 spectropolarimeter. Melting points are uncorrected. MS data were obtained under the following conditions (EI-MS and HRMS: ionization voltage, 30 eV. Negative ion FAB-MS: accelerating voltage, 2–3 kV; matrix, glycerol; collision gas, Xe). Optical rotations and CD spectra were determined for solutions in MeOH. GLC was carried out under the following operating conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m $\times$ 3 mm i.d.); FID detector; column temperature, 180 $^\circ$ C; carrier gas, N₂ 30 ml/min. For column chromatography, Merck Kieselgel 60 (230–400 mesh) and Sephadex LH-20 were used and for thin layer chromatography, precoated silica gel plates, Merck HF-254, were used. Preparative HPLC was carried out on a Waters instrument with a M 6000A pump, a U6K septumless injector, a series R-401 differential refractometer and a reversed phase ODS column (Waters μ -Bondapak-C₁₈; 7.8 mm $\times$ 30 cm) with H₂O–MeOH or H₂O–CH₃CN as eluents.

Plant Material Stems of *A. brevipedunculata* were collected at the campus of Setsunan University (Faculty of Pharmaceutical Sciences, Hirakata, Osaka, Japan) in October 1988 and identified by one of us (H.M.). A

voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

Extraction and Isolation of 1–6 The air-dried stems (2.8 kg) were extracted twice with MeOH (total 50 l) under reflux for 6 h. The combined extract was concentrated to dryness to afford the residue (550 g). A portion (440 g) of it was suspended in H₂O and the aqueous suspension was extracted successively with AcOEt (500 ml × 4), and *n*-BuOH (400 ml × 4). The residue (52.0 g) obtained from the *n*-BuOH layer was subjected to LH-20 column chromatography [LH-20 (500 ml); eluent MeOH] and a fraction (18.9 g) containing 1–6 was separated. This fraction was further purified by silical gel column chromatography (CHCl₃–MeOH as eluent) and repeated reversed-phase HPLC (H₂O–MeOH or H₂O–CH₃CN as eluents) to afford **1** (5.0 mg), **2** (2.6 mg), **3** (6.2 mg), **4** (8.3 mg), **5** (5.2 mg), and **6** (11.0 mg). The physical and spectral properties for 1–6 are as follows. Ampelopsionoside (**1**), a white powder, [α]_D –35.1° (*c*=0.11). IR (KBr) cm^{–1}: 3375, 2910, 1720, 1070, 1030. EI-MS *m/z* (%): 226 (*M*⁺ – 162, 23), 208 (*M*⁺ – 162 – H₂O, 63), 153 (95), 152 (94), 124 (100), 110 (63). Negative ion FAB-MS *m/z* (%): 387 [(*M*(C₁₉H₃₂O₈) – H)[–], 100], 225 [(*M* – H – 162)[–], 15]. ¹H- and ¹³C-NMR: given in Tables I, and II, respectively. CD (*c*=0.092) [θ]₂₀ (nm): +112 (295) (positive maximum). Ampelopsisrhamnoside (**2**), colorless oil, [α]_D –18.1° (*c*=0.04). IR (KBr) cm^{–1}: 3380, 2910, 1600, 1380, 1260, 1090, 1045. EI- and HRMS *m/z* (%): 402.190 (*M*⁺, Calcd for C₁₉H₃₀O₉ 402.189, 100), 328.153 (*M*⁺ – C₃H₆O₂, Calcd for C₁₆H₂₄O₇ 328.152, 7), 298 (11), 284 (29), 267 (9), 255.123 (*M*⁺ – H – 146, Calcd for C₁₃H₁₉O₅ 255.123, 56), 210 (90), 181 (72), 137 (93). Negative ion FAB-MS *m/z* (%): 401 [(*M* – H)[–], 100], 255 (19). ¹H- and ¹³C-NMR: given in Tables III and IV, respectively. Tachioside (**3**),⁵⁾ mp 205–208 °C (ref. 5, mp 211–213 °C), [α]_D –58.0° (*c*=0.31) [ref. 5, –55.4° (MeOH)]. The melting point, optical rotation, IR, EI-MS, and ¹H-NMR data of **3** were consistent with the published data for tachioside.⁵⁾ Isotachioside (**4**),^{5,6)} mp 191–192 °C (ref. 5, mp 195–197 °C), [α]_D –44.8° (*c*=0.36) [ref. 5, –54.5° (MeOH)]. The melting point, optical rotation, IR, EI-MS, and ¹H-NMR data of **4** were consistent with the published data for isotachioside.^{5,6)} Lyoniside (**5**),⁷⁾ mp 157–159 °C (ref. 7a, mp 164–165 °C; ref. 7f, mp 162–164 °C), [α]_D +38.9° (*c*=0.20) [ref. 7a, +26.7° (MeOH); ref. 7d, +27.3° (MeOH)]. The melting point, optical rotation, IR, EI-MS, ¹H- and ¹³C-NMR data of **5** were consistent with the published data for lyoniside.⁷⁾ 2-Phenylethyl D-rutinoside (**6**),⁸⁾ colorless amorphous solid, [α]_D –70.5° (*c*=0.55) [ref. 8, –101.2° (MeOH)]. Optical rotation, IR, EI- and negative FAB-MS, ¹H- and ¹³C-NMR data of **6** were consistent with the published data for 2-phenylethyl D-rutinoside.⁸⁾

Methanolysis of 1 A solution of **1** (1.0 mg) in 5% anhydrous HCl–MeOH (1 ml) was refluxed for 5 h. The reaction mixture was neutralized with Ag₂CO₃. The inorganic precipitate was filtered off and the filtrate was concentrated under reduced pressure to give the residue. The residue was trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide-pyridine, and subjected to GLC analysis to demonstrate the presence of methyl glucoside.

Methanolysis of 2 A solution of **2** (0.5 mg) in 5% anhydrous HCl–MeOH (1 ml) was refluxed for 5 h. The reaction mixture was worked up in the same manner as in the case of **1** and subjected to GLC analysis to demonstrate the presence of methyl rhamnoside.

References and Notes

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- 9) The configurations of 13-H₃ and 6-OH in **1** were deduced as follows. In Table I (in MeOH-*d*₄), observed *J* values between 5β-H and 4α-H [13.4 Hz (diaxial relation)] and between 5β-H and 4β-H [4.6 Hz (axial–equatorial relation)] indicate the equatorial nature of 13-H₃. The configuration of 6-OH was deduced as follows. a) In Table I, it can be seen that the signals of 2α-H and 4α-H were shifted downfield by 6-OH (1,3-diaxial relation); b) In the NOESY experiment (in pyridine-*d*₅), correlation peaks between 6-OH and 11-H₃ and 13-H₃ were observed. Thus, α-equatorial and α-axial orientations of 13-H₃ and 6-OH were respectively assigned.
- 10) With respect to the configuration of glucose in **1**, the D form may be preferable from the viewpoint of natural occurrence.
- 11) All the signals due to the side chain carbons in **1** were similar in chemical shifts (and also multiplicities) to those of analogous 9-*O*-β-D-glucopyranosyl ionones, i.e., roseoside,¹²⁾ and actinidioionoside.¹³⁾
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- 14) It was not possible from our studies to determine the absolute configuration of the C₉ chiral center on the side chain.
- 15) With respect to the configuration of rhamnose in **2**, the L form may be preferable from the viewpoint of natural occurrence. Coupling constants *J*_{1,2} (1.5 Hz) and *J*_{2,3} (3.4 Hz) (Table III) may be reasonably assigned to diequatorial (*J*_{1,2}) and equatorial–axial (*J*_{2,3}) relations for α-anomeric orientation of L-rhamnose rather than axial–equatorial (*J*_{1,2}) and equatorial–axial (*J*_{2,3}) relations for β-anomeric orientation, because, in the latter orientation, it is assumed from inspection of a Dreiding model that *J*_{1,2} and *J*_{2,3} would have the same axial–equatorial relations, so that identical *J*-values would be expected.
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