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TWO LIGNAN GLYCOSIDES FROM *VITIS THUNBERGII*

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Abstract – Two new isolariciresinol-type lignan glycosides were isolated from *Vitis thunbergii* (Vitaceae) along with some oligostilbenes. Their structures were elucidated as (+)-3α-*O*-α-L-rhamnopyranosyl-5-methoxyisolariciresinol and (–)-3α-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosylisolariciresinol, respectively, on the basis of the spectral evidence.

INTRODUCTION

In the previous papers, we reported the constituents of a cultivated vine, *Vitis vinifera* 'Kyohou'.[2] Our interests were focused on the constituents of a wild vine, *Vitis thunbergii* ("Ebizuru" in Japanese; "Ying-Yu" in Chinese) mainly distributed in Japan, Korea and China. Both the aerial part and roots of the plant have been used in folk medicine for treatment of dysentery, rheumatic disease and tuberculosis. Our study on the constituents of *V. thunbergii* led to the isolation of two new isolariciresinol-type lignan glycosides together with several known compounds including oligostilbenoids, acylglycerides, a flavonoid and a neolignan. In this paper we describe the isolation and structural elucidation of two new lignan glycosides from the aerial part of *Vitis thunbergii* (Vitaceae), collected in Aichi Prefecture of Japan.

RESULTS AND DISCUSSION

Isolation

The methanol extract of the aerial parts of *Vitis thunbergii* was successively partitioned between ethyl acetate and water, and between 1-butanol and water to give the corresponding solubles, respectively. The 1-butanol soluble fraction was chromatographed on a porous polymer (XAD-2) column to give four fractions, a water eluate, a 30% methanol eluate, a methanol eluate and an acetone eluate. The methanol eluate was successively subjected to column chromatography on silica gel, medium pressure column

chromatography (MPLC) on ODS, column chromatography on Sephadex LH-20 and preparative HPLC to give two new lignan glycosides (**1**, **2**), together with known compounds: three lignan glycosides, (+)-acviculin (**3**),[3] (–)-woonenoside XI (**4**),[4] and (+)-isolariciresinol-3α-β-D-glucopyranoside (**5**),[5] and three oligostilbenoids, (–)-α-viniferin (**8**),[6] (+)-*cis*-miyabenol C (**11**),[7] and (+)-*trans*-miyabenol C (**12**).[7] On the other hand, the ethyl acetate soluble fraction of the methanol extract gave eleven known oligostilbenoids: (+)-ε-viniferin (**6**),[8] (+)-*cis*-ε-viniferin (**7**),[9] (–)-α-viniferin (**8**),[6] (+)-ampelopsin E (**9**),[10] (+)-ampelopsin C (**10**),[11] (+)-*cis*-miyabenol C (**11**),[7] (+)-*trans*-miyabenol C (**12**),[7] (+)-viniferol D (**13**),[12] (+)-hopeaphenol (**14**),[13] (–)-isohopeaphenol (**15**),[13] (–)-vitisin B (**16**),[14] a known neolignan, (+)-(7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**17**),[15] a known flavonoid, (+)-quercimeritrin (**18**),[16] two known glycerides, (–)-1-α-linolenoyl-3-*O*-β-D-galactopyranosy glycerol (**19**),[17] and 1-*O*,3-*O*-dilinolenoly-β-D-galactopyranosyl glycerol (**20**),[18] and two known carboxylic acids, 2,5-dihydroxybenzoic acid (**21**)[19] and *trans*-4-hydroxy-3-methoxycinnamic acid (**22**).[19]

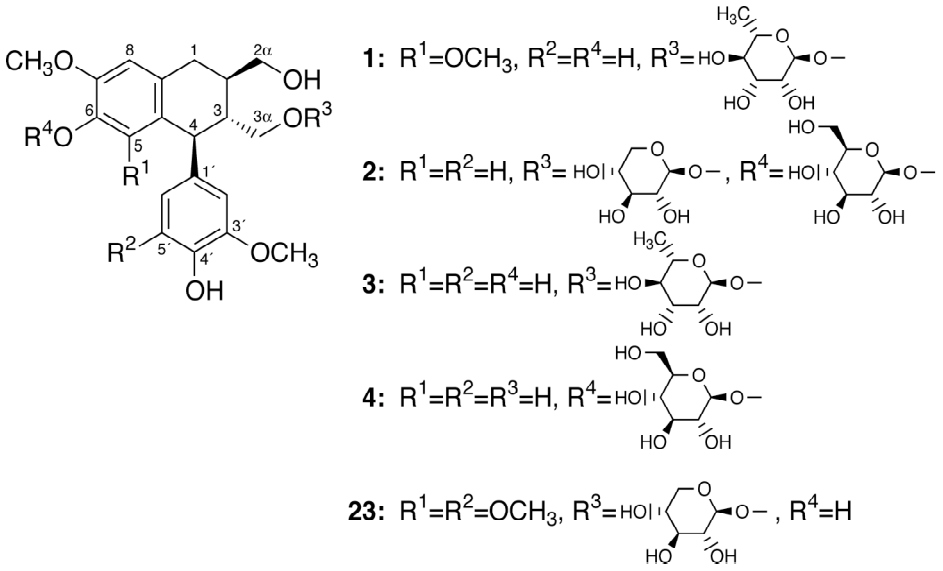

Figure 1 Lignan glycosides isolated from *Vitis thunbergii*

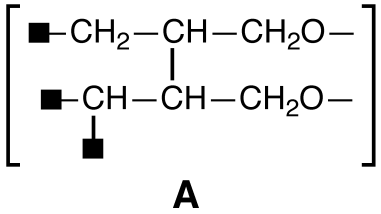

Figure 2 Partial structure **A** of compounds (**1**) and (**2**)

Structures

Compound (**1**), $[\alpha]_D$ +13.9° (MeOH, *c* 0.11) has the molecular formula $C_{27}H_{36}O_{11}$ determined by HRFAB-MS (*m/z* 537.2336 $(M+H)^+$). The data of the ^{13}C NMR spectrum were closely similar to those of the known isolariciresinol-type lignan glycosides (**3** and **23**), as shown in Table 1.[3,20] The structure of the aglycon part was characterized as follows. The ^1H NMR spectrum in methanol-d_4 of **1** exhibited signals for one set of ABX-type (1,3,4-trisubstituted) aromatic hydrogens at δ 6.63 (1H, *d*, *J* = 2.2 Hz), 6.65 (1H, *d*, *J* = 8.0 Hz), and 6.47, 1H, *dd*, *J* = 2.2, 8.0 Hz), an uncoupled aromatic hydrogen at δ 6.12 (1H, *s*), and three methoxyl groups at δ 3.32, 3.75, and 3.83 (each 3H, *s*). The H-H COSY spectrum indicated the presence of a partial structure [**A**]. Furthermore, the presence of an α-rhamnopyranosyl group was shown by the ^1H and ^{13}C NMR spectra (Table 1). These methoxyl groups are revealed to attach to C-5, C-7, and C-3´, respectively, according to the cross peaks on HMBC spectrum (C-5/5-OCH_3, C-7/7-OCH_3, and C-3´/3´-OCH_3). The position of the sugar was determined by the cross peaks between the anomeric proton [δ 4.70 (1H, *d*, *J*=1.8 Hz)] and C-3α (δ 69.6), and between H-3α [δ 3.30 (1H, *m*), 3.68 (1H, *dd*, *J*=9.6, 6.0 Hz)] and C-4 (δ 42.6) on HMBC spectrum. The relative stereochemistries among C-2, C-3 and C-4 were determined by the ROESY spectrum as follows. The cross peaks between H-3 and H-2α-b, H-4 and H-3α-a, H-4 and H-3α-b indicated the relative configurations of **1** to be $2R^*$, $3R^*$ and $4S^*$ as shown in Figure 1. Moreover, the absolute configuration of **1** was determined to be 2*R*, 3*R* and 4*S* by the comparison of the CD spectrum of **1** ($\Delta\varepsilon_{296}$ –0.09, $\Delta\varepsilon_{275}$ +1.99) with that of (+)-acviculin (**3**) ($\Delta\varepsilon_{291}$ –5.45, $\Delta\varepsilon_{273}$ +3.57) whose absolute configuration was already reported.[5] Because the absolute configuration at the 4-position of a lignan which have minus (–) peak at near 290 nm and plus (+) peak at near 270 nm on its CD spectrum was reported to be *S* at the 4-position.[5]

Compound (**2**), $[\alpha]_D$ –19.1° (MeOH, *c* 0.19) has the molecular formula $C_{31}H_{42}O_{15}$ determined by HRFAB-MS [*m/z* 655.2621 $(M+H)^+$]. The data of the ^{13}C NMR spectrum of **2** were also closely similar to those of the known isolariciresinol-type lignan glycosides (**3** and **4**), as shown in Table 1.[3,4] The structure of the aglycon part was characterized by the signals of the ^1H NMR spectrum for one set of ABX-type (1,3,4-trisubstituted) aromatic hydrogens at δ 6.79 (1H, *d*, *J* = 1.8 Hz), 6.75 (1H, *d*, *J* = 8.0 Hz), and 6.62 (1H, *dd*, *J* = 1.8, 8.0 Hz), two uncoupled aromatic hydrogens at δ 6.50 (1H, *s*) and 6.73 (1H, *s*), and two methoxyl groups at δ 3.81 and 3.82 (each 3H, *s*). Compound (**2**) also had the partial structure [**A**], as demonstrated by the H-H COSY spectrum. Furthermore, the presence of a β-xylopyranosyl and a β-glucopyranosyl groups was shown by the ^1H and ^{13}C NMR spectra. These two methoxyl groups are revealed to attach to C-7 and C-3´, respectively, according to the cross peaks on HMBC spectrum. The positions of two sugar groups were determined by the HMBC spectrum. The cross peaks between the anomeric proton of β-xylopyranose [δ 4.04 (1H, *d*, *J* = 7.7 Hz)] and C-3α (δ 69.6), and between the anomeric proton of β-glucopyranose [δ 4.37 (1H, *d*, *J* = 7.7 Hz)] and C-6 (δ 146.2) showed xylopyranose

Table 1 ^{13}C and ^{1}H NMR Spectral Data of Lignan Glycosides Isolated from *V. thunbergii.*

	1		**2**		**3**	**4**	**23**
Positions	^{13}C	^{1}H [a]	^{13}C	^{1}H [a]	^{13}C	^{13}C	^{13}C
1	33.7	2.73 (dd, 4.5, 15.1)	33.7	2.83 (2H, m)	33.6	33.4	33.9
		2.57 (dd, 11.7, 15.1)					
2	41.0	1.63 (m)	39.6	2.10 (m)	40.0	39.8	40.4
2α	66.4	3.45 (dd, 11.0, 7.0)	65.1	3.68 (dd, 11.0, 5.9)	65.3	65.8	65.9
		3.60 (dd, 11.0, 4.0)		3.73 (dd, 11.0, 3.6)			
3	46.6	2.05 (m)	45.5	1.88 (m)	45.5	47.4	46.7
3α	69.6	3.30 (m)	69.6	3.24 (dd, 9.9, 3.7)	67.9	62.3	70.9
		3.68 (dd, 9.6, 6.0)		3.98 (dd, 9.9, 2.6)			
4	42.6	4.29 (d, 5.8)	48.1	4.07 (d, 10.6)	48.3	47.8	42.9
5	147.5		119.2	6.50 (s)	117.1	118.9	148.8
6	138.9		146.2		146.1	146.2	138.8
7	148.8		148.6		149.2	148.5	147.5
8	107.9	6.57 (s)	113.3	6.73 (s)	112.4	113.2	107.7
9	130.1		132.5		128.9	132.3	130.0
10	126.3		134.9		133.9	134.8	126.4
1′	140.0		138.2		138.1	138.2	139.3
2′	113.2	6.63 (d, 2.2)	114.4	6.79 (d, 1.8)	113.4	113.9	106.8
3′	148.7		149.0		147.2	149.1	148.8
4′	145.5		146.0		145.2	146.0	134.4
5′	115.9	6.65 (d, 8.0)	116.2	6.75 (d, 8.0)	116.1	116.2	148.8
6′	121.8	6.47 (dd, 8.0, 2.2)	123.2	6.62 (dd, 8.0, 1.8)	123.2	123.3	106.8
5-OMe	60.1	3.32 (3H, s)				60.0	
7-OMe	56.7	3.83 (3H, s)	56.9	3.82 (3H, s)	56.3	56.9	56.5
3′-OMe	56.5	3.75 (3H, s)	56.6	3.81 (3H, s)	56.3	56.5	56.8
5′-OMe					56.8		
3α-	Rham		Xyl		Rham		Xyl
1″	102.0	4.70 (d, 1.8)	105.8	4.04 (d, 7.7)	102.3		105.4
2″	72.4	3.87 (dd, 3.5, 1.6)	75.0	3.18 (dd, 9.2, 7.7)	72.3		74.8
3″	72.6	3.66 (dd, 9.6, 3.3)	77.9	3.27 (dd, 8.8, 9.2)	72.5		77.9
4″	74.0	3.35 (t, 9.6)	71.3	3.45 (ddd, 10.2, 8.8, 5.5)	73.8		71.1
5″	70.1	3.53 (dq, 9.5, 6.2)	66.9	3.10 (dd, 11.4, 10.2)	70.1		66.9
				3.79 (dd, 11.4, 5.5)			
6″	17.9	1.18 (3H, d, 6.2)			17.9		
6-			Glc			Glc	
1‴			103.6	4.37 (d, 7.7)		103.4	
2‴			74.7	3.37 (dd, 9.2, 7.7)		74.6	
3‴			77.9	3.27 (t, 9.2)		77.8	
4‴			70.8	3.40 (t, 9.2)		70.7	
5‴			77.9	2.92 (ddd, 9.2, 4.0, 2.6)		77.9	
6‴			62.0	3.59 (dd, 12.1, 2.6)		61.9	
				3.65 (dd, 12.1, 4.0)			

[a] number of protons, spritting patterns, and coupling constants (Hz) are in parentheses.

and glucose to be located on C-3α and C-6, respectively. The relative stereochemistries among C-2, C-3 and C-4 were determined by the NOESY spectrum as follows. The cross peaks between H-2 and H-4, and H-3 and H-1´ indicated the relative configurations of **2** to be 2R^*, 3R^*, and 4S^* as shown in Fig. 1. The absolute configuration of **2** was elucidated in the same manner for **1** to be 2*R*, 3*R*, and 4*S* on the basis of the CD spectral data of **2** ($\Delta\varepsilon_{291}$ –5.98, $\Delta\varepsilon_{274}$ +3.54).

Glucose and rhamnose contained in the compounds of this plant are supposed to be D- and L-form, respectively, since the values of specific rotations of the lignan glycosides (**3**, **4** and **5**) obtained together with compounds (**1**) and (**2**) are identical with those reported.[3–5] So we determined the sugars of compounds (**1**) and (**2**) to be those shown on Figure 1.

EXPERIMENTAL

General

UV and IR spectra were recorded on JASCO Ubest V-560 (cell length 10 mm) and FT/IR-410 spectrophotometers, respectively. Optical rotations were measured with a JASCO P-1020 polarimeter (cell length 100 mm). ^{1}H and ^{13}C NMR spectra were recorded on JEOL ALPHA-600 (^{1}H: 600 Hz and ^{13}C: 150 MHz). Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to solvent signal (methanol-d_4: δ 3.30 and δ_C 49.0) as internal standards, respectively. HRFAB-MS were obtained with JEOL JMS HX-110 using *m*-nitrobenzylalcohol as matrix. CD spectra were recorded on CD-725. Analytical TLC was performed on silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd).

Plant material

Vitis thunbergii was collected in Toyoake City, Aichi Prefecture, Japan. The voucher specimen was deposited at the Department of Pharmacy, Meijo University.

Isolation

A fresh aerial part of *V. thunbergii* (18 kg) was extracted with MeOH (60 L) at rt for a week to yield the extract (966 g). The methanol extract was successively partitioned between ethyl acetate and water, and between 1-butanol and water to give the ethyl acetate (300 g) and 1-butanol (257 g) solubles, respectively. A part of the 1-butanol extract (140 g) was subjected to column chromatography over Amberlite XAD-2 (2 L) to give water eluate (75 g), 30% methanol eluate (9 g), methanol eluate (49 g) and acetone eluate (6 g) fractions. The methanol fraction (49 g) was chromatograohed over silica gel (500 g) using a gradient solvent system of chloroform and methanol to give 8 fractions, BM-1 – 8 [0.6 g ($CHCl_3$), 1 g (10% MeOH), 2 g (20% MeOH), 12 g (20% MeOH), 9 g (30 % MeOH), 5 g (40% MeOH), 6 g (50% MeOH) and 12 g (MeOH)]. The BM-4 eluted with 20% MeOH (12 g) was chromatographed over silica gel (200 g a gradient solvent system of chloroform and methanol) to give 10 fractions (BM-4-1 – 10). The BM-4-4

eluted with 10% MeOH (1.6 g) was further subjected to MPLC (Develosil ODS 320 x 30 mm, a gradient solvent system of chloroform and methanol) to give 9 fractions (BM-4-4-1 – 9). The BM-4-4-5 eluted with 40% MeOH (125 mg) was repeatedly subjected to HPLC (Develosil C30-UG-5, 250 x 20 mm), a mixture of methanol and water (45:55) to afford compound (**1**) (1.5 mg) and (+)-acviculin (**3**) (11.0 mg).[3] BM-4-4-7 (301 mg) was repeatedly separated by HPLC [Develosil C30-UG-5, 250 x 20mm, a mixture of methanol and water (43:57)] to afford (–)-α-viniferin (**8**) (3.0 mg),[6] (+)-*cis*-miyabenol C (**11**) (1.5 mg),[7] and (+)-*trans*-miyabenol C (**12**) (8.8 mg).[7] BM-5 eluted with 30% MeOH (9 g) was chromatographed over silica gel (200 g) using a mixture of chloroform–methanol–water (69:27:4) to give 6 fractions (BM-5-1 – 6). The BM-5-3 (5.4 g) was further separated by a combination of various chromatographies [(silica gel column chromatography (chloroform–methanol), MPLC (Develosil ODS, methanol–water) and HPLC (Develosil C30, methanol–water)) to afford (–)-woonenoside XI (**4**),[4] and (+)-isolariciresinol-3α-*O*-β-D-glucopyranoside (**5**).[5] BM-5-5 (700 mg) was further fractionated by Sephadex LH-20 using a gradient solvent system of methanol and water to give 5 fractions (BM-5-5-1 – 5). BM-5-5-1 eluted with 70% MeOH (386 mg) was subjected to MPLC (Develosil ODS 320 x 30 mm, using a gradient solvent system of methanol and water) to afford 7 fractions (BM-5-5-1-1 – 7). Finally BM-5-5-1-5 (39 mg, eluted with 30% MeOH) was separated by HPLC (Develosil C30 UG-5 250 x 20mm) using a mixture of methanol and water (34:66) to afford compound (**2**) (5.6 mg).

Compound (**1**) ((+)-3α-*O*-α-L-rhamnopyranosyl-5-methoxyisolariciresinol) $[\alpha]^{25}_{D}$ +13.9° (MeOH, *c* 0.11); a colorless amorphous solid: UV λ^{MeOH}_{max} nm (log ε) 283 (3.43), 238 (3.72); IR ν^{KBr}_{max} cm^{-1} 3400; ^{1}H NMR and ^{13}C NMR spectral data are shown in Table 1; HRFAB-MS: *m/z* 537.2334 $[M+H]^+$ (537.2336 calculated for $C_{27}H_{37}O_{11}$). CD $\Delta\varepsilon_{296}$ –0.09, $\Delta\varepsilon_{275}$ +1.99, $\Delta\varepsilon_{242}$ +8.26.

Compound (**2**) ((–)-3α-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl isolariciresinol) $[\alpha]^{25}_{D}$ –19.1° (MeOH, *c* 0.19); a colorless amorphous solid: UV λ^{MeOH}_{max} nm (log ε) 285 (3.68), 235 (4.05); IR ν^{KBr}_{max} cm^{-1} 3400; ^{1}H NMR and ^{13}C NMR spectral data are shown in Table 1; HRFAB-MS: *m/z* 655.2621 $[M+H]^+$ (655.2602 calculated for $C_{31}H_{42}O_{15}$). CD $\Delta\varepsilon_{291}$ –5.98, $\Delta\varepsilon_{274}$ +3.54, $\Delta\varepsilon_{238}$ +4.39.

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