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Inhibitory Activity against Tobacco Mosaic Virus (TMV) Replication of Pinoresinol and Syringaresinol Lignans and Their Glycosides from the Root of *Rhus javanica* var. *roxburghiana*

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Four new diepoxylignan glycosides, pinoresinol-4′-*O*-[6″-*O*-(*E*)-feruloyl]-β-D-glucopyranoside (**1**), pinoresinol-4′-*O*-[4″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside (**2**), pinoresinol-4′-*O*-[3″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside (**3**), and syringaresinol- 4′-*O*-[4″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside (**4**), together with three known compounds, pinoresinol (**5**), syringaresinol (**6**), and pinoresinol-4′-*O*-β-D-glucopyranoside (**7**), were isolated from the *n*-butanol extract of *Rhus javanica* var. *roxburghiana*, and their structures were established using various spectroscopic techniques. Three glycosides (**2**−**4**) of the lignans showed moderate inhibition of multiplication of the tobacco mosaic virus.

KEYWORDS: Anacardiaceae; *Rhus javanica*; roots; lignan; glycoside; TMV

INTRODUCTION

Rhus javanica var. *roxburghiana* (*R. semialata*) is distributed throughout the island of Taiwan. It belongs to the family of Anacardiaceae, widely distributed in tropical and subtropical areas. Some of the species of this family are famous poisonous plants, such as *Toxicodendron radicans* and *Mangifera indica* (mango), a tropical Asian evergreen tree cultivated for its edible fruit. Five species of *Rhus* are found in Taiwan: *R. ambigua*, *R. hypoleuca*, *R. javanica* var. *roxburghiana*, *R. succedanea*, and *R. sylvestris*. The roots of *R. javanica* are traditionally used in Taiwan as a folk herbal remedy for diarrhea, spermatorrhea, and malaria (*1*). To understand the unusual secondary metabolism in *Rhus* plants and to elucidate the pharmacologically active constituents of *Rhus* plants, which may be the active ingredients in some traditional Chinese medicines, we initiated studies of the chemical constituents of the roots of *R. javanica*. Among this plant's chemical constituents, steroids (*2*), flavanoids (*2*, *3*), triterpenes (*4*, *6*), and condensed tannins (*7*) have been reported previously. The MeOH extract of the root of *R. javanica* was partitioned into three soluble fractions: EtOAc, *n*-BuOH, and H_2O. The EtOAc soluble fraction gave 37 pure components, of which two exhibited significant cytotoxic activity (*8*). This paper deals with the structural elucidation of four new tetrahydrofuranic-type lignan glycosides **1**−**4** isolated from the *n*-BuOH soluble fraction. Three of these glycosides exhibited moderate activities in inhibiting multiplication of the tobacco mosaic virus (TMV).

MATERIALS AND METHODS

General Experimental Procedures. IR spectra were recorded with a Perkin-Elmer 1750 FTIR spectrometer, and the films of all samples were measured on KBr disks. Optical rotations were measured with a Jasco DIP-180 digital polarimeter spectrophotometer. ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY), HMQC, and HMBC NMR spectra were obtained using a Bruker AM-400 and a DRX-500 spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-HX 110 instrument. Chromatographic stationary phases used were RP-18 (40−60 μm, Merck), silica gel (160−200 mesh), Sephadex LH-20 (25−100 μm, Pharmacia), and MCI-gel CHP20P (75−150 μm, Mitsubishi Chemical), and high-performance liquid chromatography (HPLC) was performed on a P-230-UV-230 instrument (Dalian Elite Analytical Instruments) with a HPLC column (YMC-Pack ODS-A, S-5 μm, 250 × 10 mm). The following solvent systems were used: (a) $CHCl_3$/MeOH/H_2O (80:20:3), $CHCl_3$/MeOH/H_2O (70:30:5), and MeOH/H_2O gradient (0−100%) for the glycosides and (b) $CHCl_3$/MeOH/H_2O (7:3:1) lower-layer 9 mL plus 1 mL HOAc for the sugars. Compounds on thin-layer chromatography (TLC) were detected by spraying with 5% H_2SO_4 followed by heating. Sugars were detected by spraying with an aniline-phthalate reagent.

Plant Material. The roots of *R. javanica* var. *roxburghii* were collected from a suburb of PingTung, southern Taiwan in 1998. A

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Table 1. ^{13}C and ^{1}H NMR Spectral Data (400 and 100 MHz, in CD_3COCD_3) for **1** and **4**

	1		**4**	
no.	δ_C	δ_H	δ_C	δ_H
1	133.3		133.6	
2	110.5	6.96 (1H, d, J = 2.0 Hz)	104.5	6.67 (1H, s)
3	148.1		149.5	
4	146.1		135.6	
5	117.3	6.78 (1H, d, J = 8.0 Hz)	149.5	
6	118.9	6.87 (1H, dd, J = 8.0, 2.0 Hz)	104.5	6.67 (1H, s)
7	85.6	4.61 (1H, d, J = 4.0 Hz)	85.2	4.66 (1H, d, J = 3.4 Hz)
8	54.6	2.99 (1H, m)	54.2	2.99 (1H, m)
9	71.6	4.12 (1H, dd, J = 9.5, 3.4 Hz)	72.2	4.20 (1H, dd, J = 9.4, 3.4 Hz)
		3.73[a]		3.84[a]
3-OMe	55.7	3.81 (3H, s)	56.4	3.80 (3H, s)
5-OMe			56.4	3.80 (3H, s)
1′	136.9		133.7	
2′	109.9	6.99 (1H, d, J = 2.0 Hz)	104.4	6.67 (1H, s)
3′	146.2		151.2	
4′	149.9		139.3	
5′	115.4	6.79 (1H, d, J = 8.0 Hz)	151.2	
6′	118.4	6.80 (1H, dd, J = 8.0, 2.0 Hz)	104.4	6.67 (1H, s)
7′	86.0	4.62 (1H, d, J = 4.0 Hz)	85.2	4.66 (1H, d, J = 3.4 Hz)
8′	54.7	2.99 (1H, m)	54.3	2.99 (1H, m)
9′	71.6	4.12 (1H, dd, J = 9.5, 3.4 Hz)	72.2	4.20 (1H, dd, J = 9.4, 3.4 Hz)
		3.73[a]		3.84[a]
3′-OMe	55.8	3.83 (3H, s)	56.3	3.81 (3H, s)
5′-OMe			56.3	3.81 (3H, s)
glucose				
1″	103.7	4.91 (1H, d, J = 7.2 Hz)	102.4	5.02 (1H, d, J = 7.2 Hz)
2″	74.5	3.54[a]	75.3	3.86[a]
3″	77.2	3.78[a]	74.9	3.71[a]
4″	70.8	3.47[a]	72.2	5.06 (1H, t, J = 9.1 Hz)
5″	74.1	3.54[a]	73.0	4.01 (1H, m)
6″	63.6	4.51 (1H, dd, J = 11.6, 1.6 Hz)	63.7	4.28–4.32 (2H, m)
		4.35 (1H, dd, J = 11.6, 4.0 Hz)		
feruloyl				
1‴	126.7		127.2	
2‴	110.7	7.35 (1H, d, J = 2.0 Hz)	111.2	7.32 (1H, d, J = 1.8 Hz)
3‴	147.5		148.6	
4‴	149.4		150.0	
5‴	114.9	6.88 (1H, d, J = 8.0 Hz)	116.0	6.85 (1H, d, J = 8.0 Hz)
6‴	123.5	7.15 (1H, dd, J = 8.0, 2.0 Hz)	124.0	6.88 (1H, d, J = 8.0, 1.8 Hz)
7‴	145.2	7.61 (1H, d, J = 16.0 Hz)	146.4	7.65 (1H, d, J = 16.4 Hz)
8‴	114.9	6.42 (1H, d, J = 16.0 Hz)	115.3	6.43 (1H, d, J = 16.4 Hz)
9‴	166.6		166.8	
3‴-OMe	55.9	3.91 (3H, s)	56.3	3.84 (3H, s)
feruloyl				
1⁗			127.2	
2⁗			111.1	7.29 (1H, d, J = 1.4 Hz)
3⁗			148.5	
4⁗			150.0	
5⁗			116.0	6.84 (1H, d, J = 8.4 Hz)
6⁗			124.0	6.85 (1H, dd, J = 8.4, 1.4 Hz)
7⁗			145.9	7.60 (1H, d, J = 16.0 Hz)
8⁗			115.2	6.36 (1H, d, J = 16.0 Hz)
9⁗			166.7	
3⁗-OMe			56.3	3.88 (3H, s)

[a] Signals overlap each other.

voucher specimen (no. 191230) was deposited in the Department of Botany, National Taiwan University.

Extraction and Isolation. The dry roots of *R. javanica* (8 kg) were extracted with methanol (2 × 5 L) by steeping for 2 weeks. The extract was concentrated to dryness under reduced pressure, and the residue (700 g) was suspended in water (2.5 L) and partitioned with ethyl acetate (3 × 3 L). The water layer was subsequently extracted with *n*-butanol (3 × 3 L). The *n*-butanol extract was evaporated *in vacuo* to give a residue of 130 g. The residue was subjected to dry column chromatography on silica gel (1.0 kg) and eluted with $CHCl_3$/MeOH (10:1), producing 13 fractions. Each fraction was then separated on Sephadex LH-20 and RP-18 columns, eluted with methanol/water (10−90%), and finally purified by a silica gel column with CH_2Cl_2/EtOAc/MeOH (10:10:1) and $CHCl_3$/EtOH (10:0.5−10:2) to yield **1** (34 mg), **2** (45 mg), **3** (13 mg), **4** (36 mg), pinoresinol (**5**) (19 mg), syringaresinol (**6**) (11 mg), and pinoresinol-4′-*O*-*β*-D-glucopyranoside (**7**) (8 mg).

Compound 1. Colorless powder, $C_{36}H_{40}O_{14}$, $[\alpha]_D^{27} = -14.0$ (c = 0.43, acetone). UV (MeOH) λ_{max}nm (log ϵ): 220 (4.38), 231 (sh), 283 (4.08), 301 (4.00), 329 (4.12). FABMS *m/z:* 697 $[M + 1]^+$ (1.6%), 613 (2.1%), 577 (2.0%), 549 (2.5%), 521 (1.5%), 460 (15%), 391 (10%), 369 (6.0%), 307 (100%), 289 (49%). HRFABMS *m/z:* 697.2495 $[M + 1]^+$ calcd 697.2496 for $C_{36}H_{41}O_{14}$. IR ν_{max}(film)/cm^{-1}: 3434, 2923, 1702, 1623, 1601, 1514, 1432. ^{13}C NMR and ^{1}H NMR data are presented in **Table 1**.

Compound 2. Colorless powder, $C_{46}H_{48}O_{17}$, $[\alpha]_D^{27} = -6.9$ (c = 0.45, acetone). UV (MeOH) λ_{max}nm (log ϵ): 219 (4.28), 234 (4.02), 284 (4.06), 302 (4.04), 331 (4.16). FABMS *m/z*: 873 $[M + 1]^+$ (0.3%), 613 (1.0%), 515 (2.7%), 460 (5.0%), 391 (2.5%), 307 (41%), 289

Table 2. ^{13}C and ^{1}H NMR Spectral Data (400 and 100 MHz, in CD_3COCD_3) for **2** and **3**

	2		**3**	
no.	δ_C	δ_H	δ_C	δ_H
1	137.6		137.7	
2	110.5	6.97 (1H, d, J = 2.0 Hz)	111.3	7.00 (1H, d, J = 2.0 Hz)
3	148.1		149.7	
4	146.7		146.6	
5	115.4	6.80 (1H, d, J = 8.0 Hz)	118.1	6.84 (1H, d, J = 8.0 Hz)
6	117.9	7.14 (1H, dd, J = 8.0, 2.0 Hz)	119.5	7.18 (1H, dd, J = 8.0, 2.0 Hz)
7	86.2	4.66 (1H, d, J = 4.0 Hz)	86.5	4.64 (1H, d, J = 4.4 Hz)
8	55.2	2.99 (1H, m)	55.3	3.01 (1H, m)
9	72.2	4.20 (1H, t, J = 8.4 Hz)	72.2	4.12 (1H, dd, J = 8.5, 8.3 Hz)
		3.74[a]		3.75[a]
3-OMe	56.3	3.83 (3H, s)	56.4	3.83 (3H, s)
1′	133.9		133.9	
2′	110.5	7.02 (1H, d, J = 1.6 Hz)	110.4	6.97 (1H, d, J = 2.0 Hz)
3′	146.6		148.1	
4′	150.5		150.5	
5′	119.5	6.81 (1H, d, J = 8.4 Hz)	115.9	6.79 (1H, d, J = 8.0 Hz)
6′	119.0	7.16 (1H, d, J = 8.4, 1.6 Hz)	119.0	6.82 (1H, dd, J = 8.0, 2.0 Hz)
7′	86.5	4.68 (1H, d, J = 4.0 Hz)	86.2	4.62 (1H, d, J = 4.4 Hz)
8′	55.3	2.99 (1H, overlap)	55.2	2.98 (1H, m)
9′	72.1	4.20 (1H, t, J = 8.4 Hz)	72.1	4.16 (1H, dd, J = 8.5, 7.9 Hz)
		3.74[a]		3.76[a]
3′-OMe	56.3	3.83 (3H, s)	56.4	3.82 (3H, s)
1″	102.4	5.01 (1H, d, J = 7.2 Hz)	102.4	5.08 (1H, d, J = 7.6 Hz)
2″	75.3	3.86[a]	73.0	3.70[a]
3″	74.9	3.70[a]	78.2	5.23 (1H, t, J = 9.0 Hz)
4″	72.2	5.06 (1H, t, J = 9.6 Hz)	69.8	4.82 (1H, t, J = 9.0 Hz)
5″	73.0	4.02 (1H, m)	75.1	3.90 (1H, overlap)
6″	63.7	4.28–4.32 (2H, m)	63.9	4.52 (1H, dd, J = 12.0, 2.4 Hz)
				4.43 (1H, dd, J = 12.0, 6.0 Hz)
feruloyl				
1‴	127.2		127.3	
2‴	111.2	7.32 (1H, d, J = 1.6 Hz)	111.1	7.37 (1H, d, J = 1.6 Hz)
3‴	148.6		150.0	
4‴	150.0		148.6	
5‴	116.0	6.85 (1H, d, J = 8.0 Hz)	116.0	6.89 (1H, d, J = 7.8 Hz)
6‴	124.0	6.88 (1H, d, J = 8.0, 1.6 Hz)	124.1	7.15 (1H, d, J = 7.8, 1.6 Hz)
7‴	146.4	7.65 (1H, d, J = 16.4 Hz)	145.9	7.63 (1H, d, J = 16.0 Hz)
8‴	115.3	6.43 (1H, d, J = 16.4 Hz)	115.4	6.44 (1H, d, J = 16.0 Hz)
9‴	166.8		167.1	
3‴-OMe	56.3	3.83 (3H, s)	56.4	3.92 (3H, s)
feruloyl				
1⁗	127.2		127.2	
2⁗	111.1	7.29 (1H, d, J = 1.2 Hz)	111.1	7.36 (1H, d, J = 1.6 Hz)
3⁗	148.5		149.9	
4⁗	150.0		148.6	
5⁗	116.0	6.84 (1H, d, J = 8.4 Hz)	116.0	6.87 (1H, d, J = 8.0 Hz)
6⁗	124.0	6.85 (1H, dd, J = 8.4, 1.2 Hz)	123.9	7.14 (1H, dd, J = 8.0, 1.6 Hz)
7⁗	145.9	7.60 (1H, d, J = 16.0 Hz)	145.7	7.62 (1H, d, J = 16.0 Hz)
8⁗	115.2	6.36 (1H, d, J = 16.0 Hz)	115.4	6.43 (1H, d, J = 16.0 Hz)
9⁗	166.7		167.1	
3⁗-OMe	56.3	3.88 (3H, s)	56.3	3.92 (3H, s)

[a] Signals overlap each other.

(20%), 154 (100%), 136 (60%). HRFABMS *m/z*: 873.2962 $[M + 1]^+$ calcd 873.2969 for $C_{46}H_{49}O_{17}$. IR ν_{max}(film)/cm^{-1}: 3422, 2930, 1705, 1621, 1605, 1514, 1468. ^{13}C NMR and ^{1}H NMR data are presented in **Table 2**.

Compound 3. Colorless powder, $C_{46}H_{48}O_{17}$, $[\alpha]_D^{27} = -15.5$ (c = 1.1, acetone). UV (MeOH) λ_{max}nm (log ϵ): 222 (4.35), 284 (4.11), 300 (4.02), 331 (4.14). FABMS *m/z*: 873 $[M + 1]^+$ (1.0%), 663 (1.0%), 603 (3.0%), 577 (5.0%), 549 (4.2%), 515 (4.5%), 460 (15%), 391 (10%), 307 (100%), 289 (50%). HRFABMS *m/z:* 873.2958 $[M + 1]^+$ calcd 783.2996 for $C_{46}H_{49}O_{17}$. IR ν_{max}(film)/cm^{-1}: 3419, 2924, 1703, 1626, 1605, 1514, 1458. ^{13}C NMR and ^{1}H NMR data are presented in **Table 2**.

Compound 4. Colorless powder, $C_{48}H_{52}O_{19}$, $[\alpha]_D^{27} = -6.5$ (c = 0.51, acetone). UV (MeOH) λ_{max}nm (log ϵ): 224 (4.40), 232 (4.01), 288 (4.10), 301 (4.03), 330 (4.10). FABMS *m/z*: 933 $[M + 1]^+$ (3.0%), 756 (2.0%), 579 (5.4%), 418 (12%), 391 (20%), 369 (16%), 338 (7%), 307 (100%), 289 (45%). HRFABMS *m/z:* 933.3187 $[M + 1]^+$ calcd 933.3181 for $C_{48}H_{53}O_{19}$. IR ν_{max}(film)/cm^{-1}: 3417, 2933, 1706, 1627, 1600, 1514, 1448. ^{13}C NMR and ^{1}H NMR data are presented in **Table 1**.

Pinoresinol 5. mp 119−120 °C, $C_{20}H_{22}O_6$, $[\alpha]_D^{27} = -8.5$ (c = 0.30, acetone). FABMS *m/z*: 359 $[M + 1]^+$ (51%), 307 (100%), 289 (57%). ^{1}H NMR (CD_3COCD_3): δ 7.05 (2H, d, J = 8.0 Hz, H-5,-5′), 6.96 (2H, d, J = 2.0 Hz, H-2,-2′), 6.70 (2H, dd, J = 8.0, 2.0 Hz, H-6,-6′), 4.60 (2H, d, J = 5.0 Hz, H-7,-7′), 3.85 (6H, s, -OCH_3), 4.13 (2H, dd, J = 9.3, 7.0 Hz, H-9,-9′), 3.40 (2H, dd, J = 9.3, 3.0 Hz, H-9,-9′), 2.89 (2H, m, H-8,-8′).

Syringaresinol 6. mp 172−174 °C, $C_{22}H_{26}O_8$, $[\alpha]_D^{27} = -12.9$ (c = 0.21, acetone). IR ν_{max}(film)/cm^{-1}: 3420, 2942, 1605, 1519, 1464. FABMS *m/z*: 419 $[M + 1]^+$ (57%), 391 (35%), 338 (83%), 307 (100%), 289 (63%). ^{1}H NMR (CD_3COCD_3): δ 6.56 (4H, s, H-2,-6,-2′,-6′), 4.70 (2H, d, J = 4.3 Hz, H-7,-7′), 4.26 (2H, m, H-9,-9′), 3.89 (2H, m, H-9,-9′), 3.87 (12H, s, CH_3O-3,-5,-3′,-5′), 3.07 (2H, m, H-8,-8′).

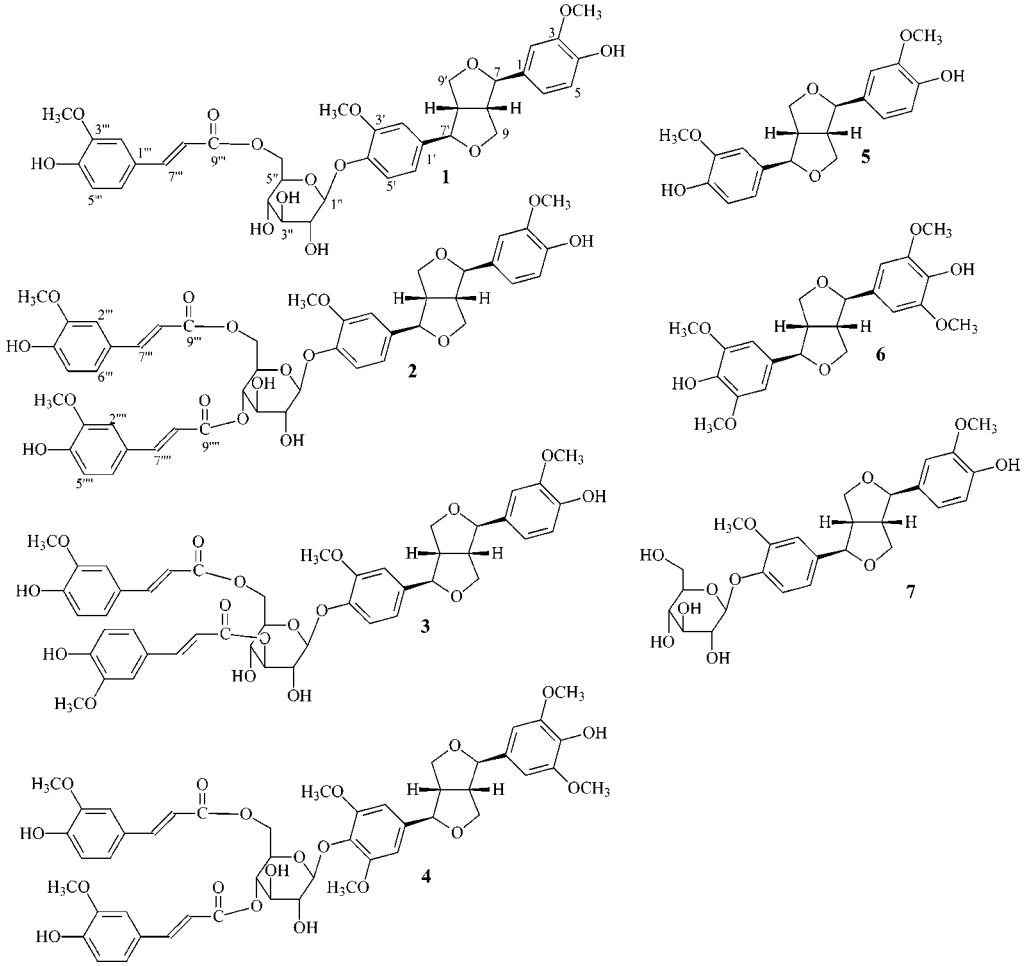

Figure 1. Compounds **1**–**7**.

Pinoresinol-4′-O-β-D-glucopyranoside 7. Colorless powder, $C_{26}H_{32}O_{11}$, $[\alpha]_D^{27} = -48.2$ ($c = 0.80$, acetone). FABMS *m/z*: 521 $[M + 1]^+$ (30%), 358 (43%), 307 (100%), 289 (64%). ^{1}H NMR (CD_3COCD_3): δ 7.12 (1H, d, $J = 8.3$ Hz), 7.04 (1H, d, $J = 1.9$ Hz), 6.98 (1H, d, $J = 1.9$ Hz), 6.89 (1H, dd, $J = 8.3$, 1.9 Hz), 6.83 (1H, d, $J = 8.3$, 1.9 Hz), 6.79 (1H, d, $J = 8.3$ Hz), 4.71 (1H, d, $J = 4.1$ Hz), 4.67 (1H, d, $J = 4.1$ Hz), 4.22 (2H, m), 3.80 (2H, m), 3.09 (2H, m), 3.84 (6H, s, -OCH_3), 4.90 (1H, d, $J = 7.4$ Hz), 3.48 (1H, m), 3.50 (1H, m), 3.39 (1H, t, $J = 9.1$ Hz), 3.62 (1H, m), 4.02 (1H, dd, $J = 9.3$, 4.6 Hz), 3.60 (1H, dd, $J = 4.6$, 2.5 Hz). ^{13}C NMR: δ 150.2 (C-4′), 148.0 (C-3), 146.8 (C-4), 146.6 (C-3′), 137.1 (C-1), 133.7 (C-1′), 119.3 (C-6), 118.9 (C-6′), 117.3 (C-5), 115.3 (C-5′), 111.2 (C-2), 110.4 (C-2′), 86.4 (C-7), 86.2 (C-7′), 72.2 (C-9), 72.1 (C-9′), 55.2 (C-8), 55.1 (C-8′), 56.3, 56.1 (2 × OCH_3), 102.3 (C_g-1), 77.7 (C_g-3), 77.6 (C_g-5), 74.5 (C_g-2), 71.1(C_g-4), 62.5 (C_g-6).

Acid Hydrolysis. A solution of compound **1** or **4** (each 10 mg) in 0.5 N H_2SO_4 (3 mL) was heated in a 95 °C water bath for 6 h. The reaction mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extract was washed with H_2O and then evaporated to dryness *in vacuo*. The aqueous layer was neutralized with Amberlite IRA 400. Compound **1** gave ferulic acid, pinoresinol (**4**) (from CH_2Cl_2 extract), and D-glucose (from aqueous solution). Ferulic acid, syringaresinol (**6**), and D-glucose were obtained from compound **4**. Nonsugar and sugar components were analyzed by silica gel HPTLC with standard authentic samples: solvent system $CHCl_3$/MeOH (10:1) for nonsugar fractions and solvent system $CHCl_3$/MeOH/H_2O (7:3:1) lower-layer 9 mL plus 1 mL of HOAc for sugar. D-glucose was also derived with thiazolidine as previously described (*9*). Monosaccharide was detected by gas chromatography (GC) under the following conditions: column, Supelco SPB-1 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R, L-glucose (13.3 min), D-glucose (13.8 min); d-glucose was detected from **1** and **4**.

Mild Alkaline Hydrolysis of Compounds 1 and 4. Each compound (5 mg) was dissolved in 0.5% NaOH (2 mL) at room temperature with stirring for 5 h. H_2O (5 mL) was added to the reaction mixture, which was acidified with excess Amberlite IRA 120 B overnight. The filtrate was subjected to RP-18 chromatography. Ferulic acid and compound **7** were obtained from compound **1,** and compound **4** yielded ferulic acid and syringaresinol-*O*-*β*-glucopyranoside (*10*).

Screening Compounds 1−7 and Indirect Enzyme-Linked Immunosorbent Assay (ELISA) Procedure. The inhibitory activity against TMV replication of each sample was determined as previously reported (*11*). *Nicotiana tabacum* cv. K_{326} was cultivated in a glasshouse without pests. Different compounds were weighed precisely with an electrobalance, dissolved in a small quantity of dimethyl sulfoxide (DMSO), and diluted with water to provide solutions having a concentration of 0.2 mg/mL; all solutions were in Petri dishes.

RESULTS AND DISCUSSION

Residue from the methanol extract of the root of *R. javanica* was suspended in water and partitioned with EtOAc and *n*-BuOH, successively. The *n*-BuOH extract was purified as described above by extraction and isolation. Four new glycosides (**1**−**4**) were isolated along with three known compounds, identified by reported NMR data. The three known compounds were pinoresinol (**5**) (*12*), syringaresinol (**6**) (*13*), and pinoresinol-4′-*O*-*β*-D-glucopyranoside (**7**) (*14*) (**Figure 1**).

Compound **1** gave the molecular formula $C_{36}H_{40}O_{14}$ based on the HRFABMS ion peak at *m/z* 697.2495 $[M + 1]^+$. The IR spectrum showed a conjugated ester carbonyl (1702 cm^{-1}), a double bond (1623 cm^{-1}), and an aromatic ring (1601 and 1514 cm^{-1}). Acid hydrolysis of **1** afforded three products which were

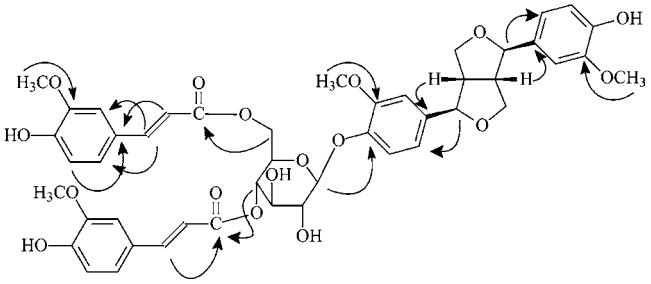

Figure 2. Key HMBC correlations (→) of **2**.

identified as D-glucose, pinoresinol, and ferulic acid. The ^{1}H NMR spectrum of **1** displayed the following signals: three sets of ABX-type aromatic proton signals at δ 6.96 (1H, d, $J = 2.0$ Hz), 6.87 (1H, dd, $J = 8.0$, 2.0 Hz), 6.78 (1H, d, $J = 8.0$ Hz); δ 6.99 (1H, d, $J = 2.0$ Hz), 6.80 (1H, dd, $J = 8.0$, 2.0 Hz), 6.79 (1H, d, $J = 8.0$ Hz); and δ 7.35 (1H, d, $J = 2.0$ Hz), 7.15 (1H, dd, $J = 8.0$, 2.0 Hz), 6.88 (1H, d, $J = 8.0$ Hz); two *trans*-double bond proton signals at δ 7.61 (1H, d, $J = 16.0$ Hz), 6.42 (1H, d, $J = 16.0$ Hz); an anomeric proton signal at δ 4.91 (1H, d, $J = 7.2$ Hz); and three methoxyl signals at δ 3.91 (3H, s), 3.83 (3H, s), 3.81 (3H, s). The ^{13}C NMR spectrum exhibited one carbonyl carbon signal at δ 166.6, two sp^2 carbon signals at δ 145.2, 114.9, nine methines, nine quaternary aromatic carbon signals, six oxygenated carbon signals due to the sugar moiety along with the aliphatic signals at δ 86.0, 85.6, 54.7, 54.6, 71.6 × 2 due to the aglycone moiety (see **Table 1**). Thus, compound **1** was believed to consist of an aglycone, one monosaccharide, and an (*E*)-feruloyl group. Concerning the aglycone moiety, the ^{13}C NMR spectrum showed 20 carbon signals including 12 aromatic carbon signals, 6 aliphatic carbon signals, and two methoxyl carbon signals. Based on the above evidence, the aglycone of **1** was deduced to be a lignan derivative. Because the six carbon signals were symmetrical signals in the aliphatic part of aglycone (δ 86.0, 85.6, 54.7, 54.6, 71.6 × 2) and four carbons were bonded to oxygen, this lignan of aglycone should be a bifuranoid lignan. (−)-Pinoresinol was identified by acidic hydrolysis. It exhibited negative specific rotation that is the same as that in compound **5**.

Ferulic acid and compound **7** (*14*) were obtained from compound **1** by basic hydrolysis. This evidence suggests that the glucosyl unit with C-1″ is linked to C-4′ by an ether linkage. Finally, the feruloyl moiety was proposed to be located at position C-6″ of glucose with H-6″ (δ 4.51 and 4.35 with the ABX system) presenting at a lower field shift and exhibiting HMBC correlation with the ester carbonyl carbon (δ 166.6). Therefore, compound **1** was elucidated to be pinoresinol-4′-*O*-[6″-*O*-(*E*)-feruloyl]-β-D-glucopyranoside.

Compounds **2** and **3** were isomers, and their NMR data were very similar. The only difference was the two esters' location. They gave the same quasi-molecular ion peak at *m*/*z* 873 $[M + 1]^+$ in FABMS, and HRFABMS analysis revealed the molecular formula to be $C_{46}H_{48}O_{17}$. The IR and UV spectra indicated the presence of a conjugated ester carbonyl, double bond, and aromatic rings. The ^{13}C and ^{1}H NMR spectra revealed the presence of a β-glucopyranosyl moiety, with pinoresinol as the aglycone, and two feruloyl moieties. The only difference between **2** and **3** was that the two feruloyl moieties were linked at different positions in the glycoside (see **Figure 1**). The HMBC spectrum (see **Figure 1**) of **2** exhibited key correlations between the two feruloyl moieties and the sugar, from H-6″ (δ 4.28−4.32) to C-9‴ (δ 166.8), from H-4″ (δ 5.06) to C-9⁗ (166.7), and from H-1″ (δ 5.01) to C-4′ (δ 150.5). The NOESY correlation between δ 4.28−4.32 and δ 5.06 clearly showed that δ 5.06 is assigned as H-4″. These correlations indicated that two feruloyl groups are linked to C-4 and C-6, and the sugar is located at C-4′ of the aglycone. However, the coupling constants of H-1″ (d, $J = 7.2$ Hz) and H-4″ (t, $J = 9.6$ Hz) were coincident with the glucosyl moiety. The HMBC spectrum of **3** gave significant correlations between H-6″ (δ 4.52, 4.43) and C-9‴ (δ 167.1), between H-3″ (δ 5.23) and C-9⁗ (δ 167.1), and between H-1″ (δ 5.08) and C-4′ (δ 150.5), with the following COSY correlations: H-2″ (δ 3.70)/H-1″, H-3″ (δ 5.23); H-4″ (δ 4.82)/H-5″ (δ 3.90), H-3″. In addition, a NOESY correlation between H-5″ and H-3″ indicated a second feruloyl linkage to C-3″. Based on the above evidence, compound **2** was assigned as pinoresinol-4′-*O*-[4″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside, and compound **3** was determined to be pinoresinol-4′-*O*-[3″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside.

The molecular formula of **4** was determined to be $C_{48}H_{52}O_{19}$ by HRFABMS (*m*/*z* 933.3187 $[M + 1]^+$). The IR and UV spectra indicated the presence of conjugated ester carbonyl (1706 cm^{-1}), double bond (1627 cm^{-1}), and aromatic rings (1600, 1514 cm^{-1}). The ^{13}C and ^{1}H NMR spectra showed the presence of a β-glucopyranosyl moiety, two feruloyl moieties, two 1,3,4,5-tetrasubstituted symmetrical benzene signals (δ 6.67, 2H), two oxygenated methylene signals at δ 4.20 (2H, dd, $J =$ 9.4, 3.4 Hz), 3.84 (2H, overlapping), and four methine signals including two oxygen-bearing methines [δ 4.66 (2H, d, $J =$ 3.4 Hz, H-7,-7′), 2.99 (2H, m, H-8,-8′)], along with six methoxy signals at δ 3.81 (6H, s) and 3.80 (6H, s) (see **Table 1**). By comparison of the above ^{1}H NMR data with those of syringaresinol-*O*-β-glucopyranoside (*15*), compound **4** was determined to be a derivative of syringaresinol-*O*-β-glucopyranoside with two additional feruloyl moieties. Mild alkaline hydrolysis of compound **4** produced $\{[\alpha]_D^{27} = -26.3$ ($c = 0.24$, MeOH)$\}$, which was identified as syringaresinol-*O*-β-D-glucopyranoside. Compound **4** displayed significant HMBC correlations between H-6″ (δ 4.28) and C-9‴ (δ 166.8), between H-4″ (δ 5.06) and C-9⁗ (δ 166.7), and between H-1″ (δ 5.02) and C-4′ (δ 139.3), and a NOESY correlation of H-6″/H-4″. Therefore, the structure of **4** was determined to be syringaresinol-4′-*O*-[4″,6″-*O*-(*E*)-diferuloyl]-β-D-glucopyranoside.

The inhibitory activities against TMV of compounds **1**−**7** were evaluated *in vitro*. Of the seven compounds tested at a concentration of 0.2 mg/mL, compounds **1**−**4** showed higher inhibitory activity against TMV replication than the other compounds, with 34.3, 50.3, 47.7, and 56.1% inhibition, respectively. The inhibitory effects of compounds **1**−**4** at several concentrations were tested, and the EC_{50} values of compounds **2**−**4** were determined to be 0.19, 0.24, and 0.18 mg/mL, respectively.

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