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Lignans from Bark of *Fraxinus mandshurica* var. *japonica* and *F. japonica*

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Three new lignans, (+)-fraxiresinol [(1*S*,2*R*,5*R*,6*S*)-1-hydroxy-2-(3,5-dimethoxy-4-hydroxyphenyl)-6-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane] (**3**), (+)-1-hydroxysyringaresinol (**4**) and (+)-1-hydroxypinoresinol-4'- β -D-glucoside (**8**), and five known lignans, (+)-pinoresinol (**1**), (+)-1-hydroxypinoresinol (**2**), (-)-olivil (**5**), (+)-cyclo-olivil (**6**) and (+)-pinoresinol- β -D-glucoside (**7**), were isolated from the bark of *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM and the bark of *F. japonica* BLUME (Oleaceae).

Their structures were elucidated on the basis of spectroscopic analysis and chemical evidence.

Keywords—*Fraxinus mandshurica* var. *japonica*; *Fraxinus japonica*; Oleaceae; lignan; (+)-fraxiresinol; (+)-1-hydroxysyringaresinol; (+)-1-hydroxypinoresinol; (+)-pinoresinol; (-)-olivil; (+)-cyclo-olivil

Fraxinus mandshurica RUPR. var. *japonica* MAXIM (Oleaceae) is widely distributed in Hokkaido, the northern part of Japan. In China, the dried bark of *F. mandshurica* is sometimes used as a substitute for the Chinese crude drug "qin pi (Cortex Fraxini, 秦皮)".¹⁾ Further, the dried bark of *F. japonica* BLUME, which is on the market as "shinpi (秦皮)" in Japan, has been used since olden times as a diuretic, an antifebrile, an analgesic and an anti-rheumatic.²⁾

Various coumarins, *i.e.* fraxinol, fraxetin, fraxin and mandshurin from *F. mandshurica* var. *japonica*^{3a,b)} and fraxetin, esculetin, fraxin and esculin from *F. japonica*,^{4a-c)} have been isolated from these barks so far. Among them, esculin and esculetin are known to be active principles of "shinpi".⁵⁾ Recently, compounds which show anti-inflammatory and antiplatelet-aggregating activities were also reported from the bark of *F. japonica*.⁶⁾

Thus, our interest has been directed to the investigation of the constituents of these *Fraxinus* barks. This paper describes the isolation of three new lignans, **3**, **4** and **8**, along with five known lignans, (+)-pinoresinol (**1**), (+)-1-hydroxypinoresinol (**2**), (-)-olivil (**5**), (+)-cyclo-olivil (**6**) and (+)-pinoresinol- β -D-glucoside (**7**), from the bark of *F. mandshurica* var. *japonica* and the bark of *F. japonica*, and their structure elucidation on the basis of spectroscopic analysis and chemical evidence. The extraction and separation were carried out as described in Experimental.

Lignans **1** and **2** were identified as (+)-pinoresinol and (+)-1-hydroxypinoresinol, respectively, by direct comparison with authentic samples.^{7,8)}

The lignan **3** was obtained as an amorphous powder, C₂₁H₂₄O₈, $[\alpha]_D^{25} + 29.3^\circ$ (chloroform). The infrared (IR) spectrum of **3** suggested the presence of aromatic rings (1615 and 1510 cm⁻¹). The ultraviolet (UV) spectrum of **3** showed absorption maxima at 232.3 and 279.8 nm. The bathochromic shift of the absorption maxima in the presence of base was very similar to that of (+)-medioresinol (**3c**).⁹⁾ The proton nuclear magnetic resonance (¹H-NMR) spectrum of **3** exhibited signals at δ 3.85 (9H, s) due to aromatic methoxy protons and at δ 6.58 (2H, s) and 6.73—7.03 (3H, m) due to aromatic protons, and the signals of other protons

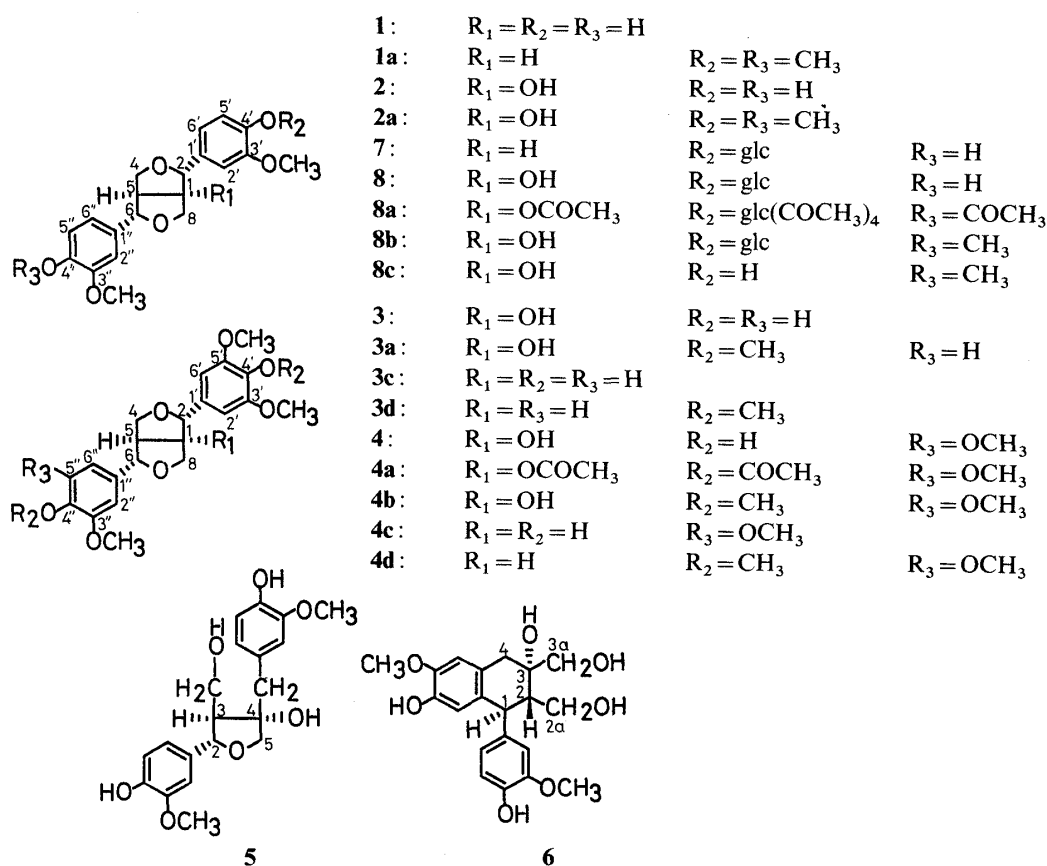


Chart 1

resembled those of **2**. Methylation of **3** with diazomethane gave **3a** as an amorphous powder, $C_{23}H_{28}O_8$, $[\alpha]_D^{25} + 13.1^\circ$ (ethanol). The 1H -NMR spectrum of **3a** showed the presence of five aromatic methoxy groups (δ 3.84). The UV spectrum of **3a** was very similar to that of (+)-medioresinol dimethyl ether (**3d**).⁹⁾

These data indicated that **3** contains a 2,6-diaryl-1-hydroxy-3,7-dioxabicyclo[3.3.0]octane ring and that the aryl groups of **3** consist of a guaiacyl unit and a syringyl unit.

The carbon-13 nuclear magnetic resonance (^{13}C -NMR) spectra of **3** and **3a** were compared with those of known lignans, **1**, **1a**, **2**, **2a**, **3c** and **3d**. The ^{13}C -NMR data of **3** indicated that **3** bears stereochemically the same structural skeleton as **2**. The differences of chemical shifts at the C-1', C-2', C-3', C-5' and C-6' carbons between **3** and **3c** ($\Delta\delta$ -4.3, +1.9, -0.5, -0.5 and +1.9) and between **3a** and **3d** ($\Delta\delta$ -4.4, +1.9, -0.6, -0.6 and +1.9), which are due to the effect of the alcoholic hydroxyl group at the C-1 position of **3** and **3a**, clearly indicated that the aryl group at the C-2 position of **3** is the syringyl unit and the aryl group at the C-6 position of **3** is the guaiacyl unit. The mass spectral (MS) fragmentation patterns of **3** and **3a** were also in good agreement with the results of ^{13}C -NMR analysis.

Chemical evidence for the structure **3** was obtained as follows. The sodium-ammonia reduction of **3a** afforded **3b** as a colorless syrup, $C_{23}H_{32}O_8$, $[\alpha]_D^{22} - 15.4^\circ$ (chloroform). All the spectral data for **3b** were in good agreement with those for the triol **3b** which was obtained by the reduction of hydroxythujaplicatin trimethyl ether (di-*O*-methylhydroxythujaplicatin methyl ether)¹⁰⁾ with lithium aluminum hydride.

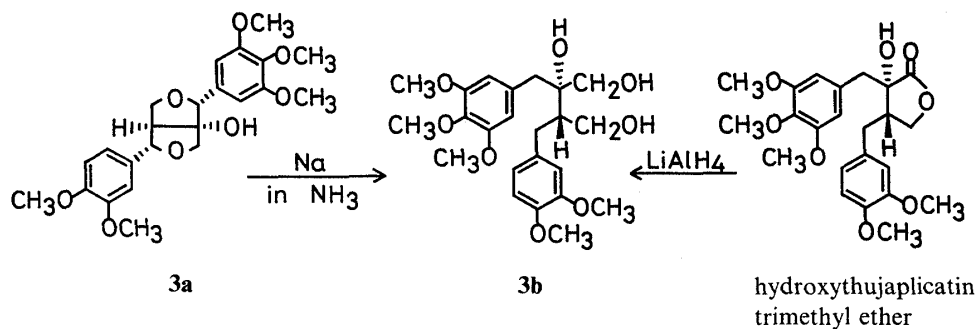
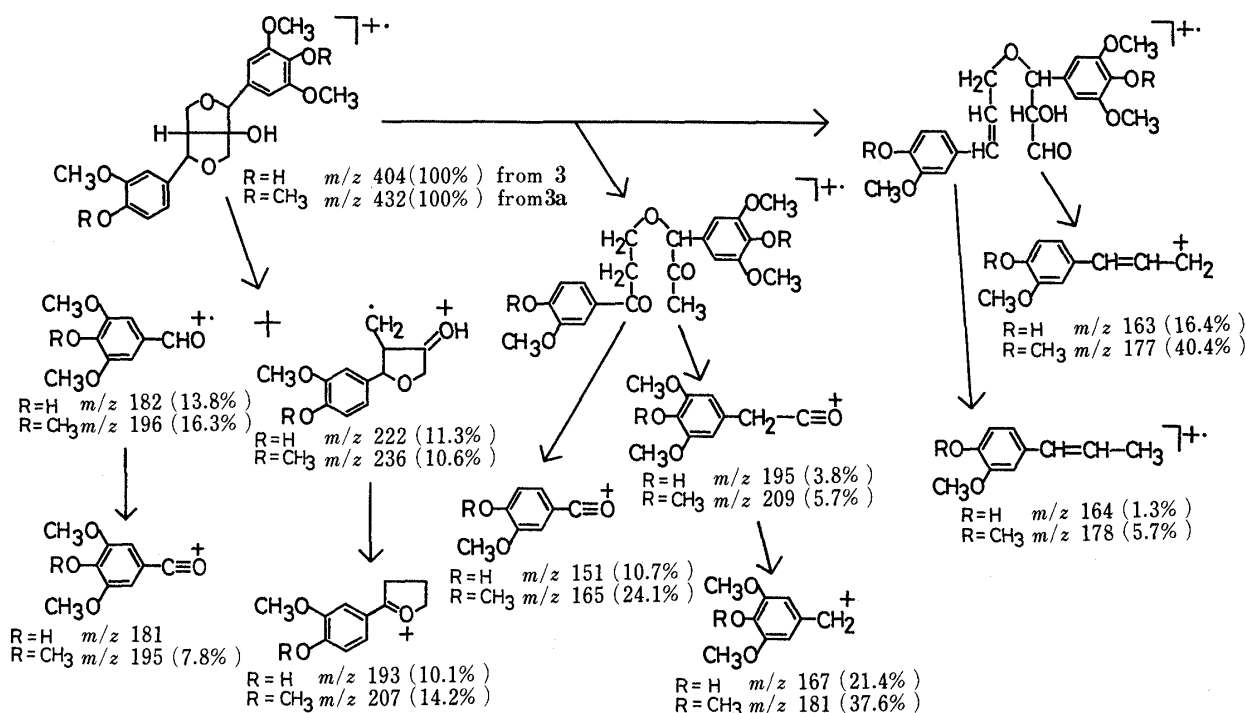
Thus, the structure of **3** has been established as (1*S*, 2*R*, 5*R*, 6*S*)-1-hydroxy-2-(3,5-dimethoxy-4-hydroxyphenyl)-6-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane, and this compound has been designated as (+)-fraxiresinol.

The lignan **4** was obtained as a colorless crystalline powder, $C_{22}H_{26}O_9$, mp 95–97 °C,

TABLE I. ^{13}C -NMR Chemical Shifts^{a)}

	2	1	$\Delta\delta$ (2-1)	2a	1a	$\Delta\delta$ (2a-1a)	3	3c	$\Delta\delta$ (3-3c)	3a	3d	$\Delta\delta$ (3a-3d)	4	4c	$\Delta\delta$ (4-4c)	4b	4d	$\Delta\delta$ (4b-4d)
C-1	91.0	53.6		91.0	53.7		91.1	53.6		91.2	53.7		91.0	53.6		91.2	53.7	
C-5	60.8	53.6		60.8	53.7		60.7	53.4		60.8	53.5		60.8	53.6		60.8	53.7	
C-4	70.2	70.9		70.2	71.0		70.2	71.0		70.3	71.1		70.2	71.0		70.4	71.2	
C-8	74.7	70.9		74.7	71.0		74.6	70.8		74.6	71.0		74.7	71.0		74.7	71.2	
C-2	87.1	85.2		86.9	85.0		87.2	85.2		87.0	85.0		87.1	85.3		86.9	85.0	
C-6	85.4	85.2		85.1	85.0		85.3	85.1		85.0	84.8		85.4	85.3		85.1	85.0	
C-1'	128.1	132.3	-4.2	129.7	134.0	-4.3	127.1	131.4	-4.3	132.8	137.2	-4.4	127.1	131.5	-4.4	132.8	137.2	-4.4
C-1''	132.3	132.3		133.9	134.0		132.3	132.2		133.9	133.8		131.4	131.5		137.1	137.2	
C-2'	112.3	110.5	+1.8	111.9	110.0	+1.9	105.5	103.6	+1.9	105.0	103.1	+1.9	105.5	103.7	+1.8	105.0	103.1	+1.9
C-2''	110.8	110.5		110.3	110.0		110.8	110.4		110.3	109.9		104.1	103.7		103.4	103.1	
C-3'	146.9	147.6	-0.7	148.1	148.9	-0.8	147.4	147.9	-0.5	152.2	152.8	-0.6	147.3	147.9	-0.6	152.2	152.8	-0.6
C-3''	147.5	147.6		148.7	148.9		147.8	147.5		148.7	148.7		147.8	147.9		152.7	152.8	
C-4'	145.9	145.9		148.2	148.3		134.9	134.8		136.8	136.6		134.9	134.9		136.8	136.7	
C-4''	145.9	145.9		148.2	148.3		145.9	145.9		148.2	148.1		134.9	134.9		136.8	136.7	
C-5'	114.5	115.2	-0.7	111.2	111.7	-0.5	147.4	147.9	-0.5	152.2	152.8	-0.6	147.3	147.9	-0.6	152.2	152.8	-0.6
C-5''	115.1	115.2		111.7	111.7		115.1	115.1		111.7	111.6		147.8	147.9		152.7	152.8	
C-6'	120.2	118.6	+1.6	119.7	118.1	+1.6	105.5	103.6	+1.9	105.0	103.1	+1.9	105.5	103.7	+1.8	105.0	103.1	+1.9
C-6''	118.8	118.6		118.3	118.1		118.8	118.5		118.4	118.1		104.1	103.7		103.4	103.1	
OCH ₃	55.6	55.6		55.4	55.4		55.6	55.6		55.4	55.4		55.9	56.0		55.7	55.7	
							55.9	55.9		55.7	55.8					59.8	59.8	
										59.8	59.8							

a) The spectra were taken in micro cells with a JNM-FX 60 spectrometer (15.00 MHz) in DMSO- d_6 with TMS as an internal reference.



$[\alpha]_D^{24.5} + 24.6^\circ$ (chloroform). The IR spectrum of **4** suggested the presence of aromatic rings (1615 and 1515 cm^{-1}). The absorption maxima in the UV spectrum of **4** and its bathochromic shift in the presence of base were very similar to those of (+)-syringaresinol (**4c**).^{9,11} The $^1\text{H-NMR}$ spectrum of **3** exhibited signals at δ 3.85 (12H, s) due to aromatic methoxy protons and at δ 6.60 (2H, s) and 6.62 (2H, s) due to aromatic protons. Acetylation of **4** with acetic anhydride-pyridine gave **4a** as a colorless crystalline powder, $\text{C}_{28}\text{H}_{32}\text{O}_{12}$, mp $81\text{--}83^\circ\text{C}$, $[\alpha]_D^{25} + 12.0^\circ$ (chloroform). The $^1\text{H-NMR}$ spectrum of **4a** showed the presence of an alcoholic acetoxyl group (δ 1.70), two phenolic acetoxyl groups (δ 2.30 and 2.32) and four aromatic methoxy groups (δ 3.79). Methylation of **4** with diazomethane gave **4b** as an amorphous powder, $\text{C}_{24}\text{H}_{30}\text{O}_9$, $[\alpha]_D^{22} + 11.9^\circ$ (chloroform). The $^1\text{H-NMR}$ spectrum of **4b** showed the presence of six aromatic methoxy groups (δ 3.84). The UV spectrum of **4b** was very similar to that of the known compound (+)-syringaresinol dimethyl ether (**4d**).^{9,11} The oxidation of **4b** with potassium permanganate gave only 3,4,5-trimethoxybenzoic acid. These data suggest that **4** bears a marked structural resemblance to **2** and **3**, and that the aryl groups of **4** are syringyl units.

In the $^{13}\text{C-NMR}$ spectrum, the differences of chemical shifts at the C-1', C-2', C-3', C-5' and C-6' carbons between **4** and **4c** ($\Delta\delta$ -4.4 , $+1.8$, -0.6 , -0.6 and $+1.8$) and between **4b**

TABLE II. Molecular Optical Rotation Differences

	$[\alpha]_D$ (°)	$[M]$ (°)	$\Delta [M]$ (°)
4	+24.6	+106.8	-153.2
4c	+62.2	+260.0	
2	+39.0	+145.9	-131.6
1	+77.5	+277.5	
3	+29.3	+118.4	-104.3
3c	+57.4	+222.7	

and **4d** ($\Delta\delta$ -4.4, +1.9, -0.6, -0.6 and +1.9) supported the view that the aryl groups at both the C-2 and C-6 positions of **4** are syringyl units.

With regard to the problem of the absolute configuration of **4**, a comparison of the molecular optical rotation differences between **4** and **4c** with those between the known compounds, **2** and **1**, and **3** and **3c** suggested that **4** has the same absolute configuration as **2** and **3**. Consequently, the structure of **4** has been established as (1*S*, 2*R*, 5*R*, 6*S*)-1-hydroxy-2,6-bis(3,5-dimethoxy-4-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane. This compound has been designated as (+)-1-hydroxysyringaresinol.

Lignans **5** and **6** were identified as (-)-olivil and (+)-cyclo-olivil, respectively, by direct comparison with authentic samples. The lignan **7** was obtained as an amorphous powder, C₂₀H₂₂O₆, mp 107–109 °C, $[\alpha]_D^{20}$ +8.6° (ethanol). The enzymatic hydrolysis of **7** gave **1** and D-glucose. The lignan **7** was confirmed to be (+)-pinioresinol- β -D-glucoside by direct comparison with authentic sample.⁷⁾

The lignan **8** was obtained as colorless plates, C₂₆H₃₂O₁₂, mp 127–129 °C, $[\alpha]_D^{23}$ -9.3° (methanol). The absorption maxima in the UV spectrum of **8** and its bathochromic shift in the presence of base were very similar to those of **7**. Acetylation of **8** with acetic anhydride-pyridine gave **8a** as a colorless syrup, C₃₈H₄₄O₁₈, $[\alpha]_D^{20}$ -6.3° (ethanol). The ¹H-NMR spectrum of **8a** showed the presence of five alcoholic acetoxy groups (δ 1.67, 2.03 and 2.10), a phenolic acetoxy group (δ 2.33) and two aromatic methoxy groups (δ 3.83 and 3.87). The enzymatic hydrolysis of **8** gave **2** and D-glucose. The ¹³C-NMR spectrum of **8** also revealed that **8** is a monoglucoside of **2**. Methylation of **8** with diazomethane gave **8b** as an amorphous powder, C₂₇H₃₄O₁₂·1.5H₂O, mp 117–120 °C, $[\alpha]_D^{23}$ -1.3° (ethanol). The enzymatic hydrolysis of **8b** gave **8c** as an amorphous powder, C₂₁H₂₄O₇, $[\alpha]_D^{23}$ +37.9° (chloroform) and D-glucose. Compound **8c** was identified as (+)-1-hydroxypinioresinol 4'-*O*-methyl ether by direct comparison with an authentic sample.⁸⁾ Therefore, as regards the position of the glucose linkage in **8**, these data indicate that D-glucose is attached to the 4'-*O*-position of **2**. Thus, the structure of **8** has been established as (+)-1-hydroxypinioresinol-4'- β -D-glucoside.

Our results show that there is no difference between the lignans contained in the barks of *F. mandshurica* var. *japonica* and *F. japonica*, despite the difference in the coumarins contained.

In regard to the biological activity of the isolated lignans, lignans **1** and **7** showed high inhibitory activity against cyclic adenosine monophosphate (cAMP)-phosphodiesterase *in vitro* (IC₅₀ ($\times 10^{-5}$ M): 7.5 and 14.2).¹²⁾ Weinryb *et al.* reported that a considerable number of therapeutic agents used as antipsychotics, antianxiety agents, antihypertensives and so on showed inhibitory effects against phosphodiesterase.¹³⁾ Thus, these lignans might possess some pharmacological activity.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The

following instruments were used: optical rotations, Yanaco OR-50D; UV spectra, Shimadzu UV-210; IR spectra, Shimadzu IR-400 and Hitachi 270-30; circular dichroism (CD) curves, Jasco J-40; ^1H -NMR spectra, JEOL JNM-PMX 60 and Hitachi R-40 with tetramethylsilane (TMS, $\delta=0$) as an internal reference; ^{13}C -NMR spectra, JEOL JNM-FX 60, equipped with a JEC-980 computer; MS, Hitachi RMU-7L and Shimadzu LKB-9000. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; t, triplet; q, quartet; sh, shoulder. Precoated thin-layer chromatography (TLC) plates, Silica gel 60_{F254} (Merck), were used for TLC and preparative TLC. The spots were detected by spraying the plates with 10% H_2SO_4 soln. and heating. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation—Dry powdered bark of *Fraxinus mandshurica* var. *japonica* (4.3 kg), collected in December 1979 at our University, Hokkaido, Japan, were extracted four times with hot MeOH. The MeOH solution was concentrated to a small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with ether, CHCl_3 and BuOH.

The ether layer was evaporated to dryness. The ether extract (15.7 g) was subjected to column chromatography; elution was carried out with a CHCl_3 -AcOEt solvent system with gradually increasing proportions of AcOEt. The fractions were monitored by TLC developed with CHCl_3 -AcOEt (1:2). The fractions (100 ml each) showing a TLC spot at R_f 0.59 were concentrated, and the residue was purified by preparative TLC using CHCl_3 -AcOEt (1:1) to give 133.7 mg of **1**. When treated in the same way as described for **1**, the fractions showing TLC spots at R_f 0.37, 0.30, 0.24, 0.19 and 0.09 gave 121.2 mg of **2**, 141.0 mg of **3**, 94.9 mg of **4**, 115.6 mg of **5** and 65.3 mg of **6**, respectively.

The CHCl_3 layer was evaporated to dryness. The CHCl_3 extract (20.2 g) was subjected to column chromatography; elution was carried out with a CHCl_3 -EtOH solvent system with gradually increasing proportions of EtOH. The fractions were monitored by TLC developed with CHCl_3 -EtOH (4:1). The fractions (100 ml each) showing a TLC spot at R_f 0.35 were concentrated, and the residue was purified by preparative TLC using CHCl_3 -EtOH (4:1) to give 53.4 mg of **7**.

The BuOH layer was evaporated to dryness. The BuOH extract (170.7 g) was subjected to column chromatography; elution was carried out with a CHCl_3 -EtOH solvent system with gradually increasing proportions of EtOH. The fractions were monitored by TLC developed with CHCl_3 -EtOH (4:1), no TLC spot of lignans was detected.

Dry powdered bark (3.5 kg) of *Fraxinus japonica* was treated in the same manner as described for *Fraxinus mandshurica* var. *japonica*. The ether extract (16.7 g) gave 108.0 mg of **1**, 38 mg of **2**, 29.2 mg of **3**, 29 mg of **5** and 8 mg of **6**. The CHCl_3 extract (14.4 g) gave 89.3 mg of **7**. The BuOH layer was evaporated to dryness. The BuOH extract (40.0 g) was subjected to column chromatography; elution was carried out with a CHCl_3 -EtOH solvent system with gradually increasing proportions of EtOH. The fractions were monitored by TLC developed with CHCl_3 -EtOH (4:1). The fractions (100 ml each) showing a TLC spot at R_f 0.15 were concentrated, and the residue was purified by preparative TLC using CHCl_3 -MeOH (4:1) to give 43.0 mg of **8**.

(+)-Pinoresinol (1)—Colorless prisms, mp 119–120°C, $[\alpha]_D^{21} + 77.5^\circ$ ($c=0.26$ in CHCl_3), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 232.0 (4.14), 281.0 (3.75). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3545 (OH), 1610, 1510 (arom. C=C). MS m/z : 358 (M^+ , $\text{C}_{20}\text{H}_{22}\text{O}_6$). ^1H -NMR (in CDCl_3) δ : 2.87–3.33 (2H, m, $\text{C}_{1,5}\text{-H}$), 3.87 (6H, s, $2 \times \text{OCH}_3$), 3.56–4.46 (4H, m, $\text{C}_{4,8}\text{-H}$), 4.73 (2H, d, $J=5$ Hz, $\text{C}_{2,6}\text{-H}$), 6.65–7.05 (6H, m, arom. H). The identity of this compound was confirmed by direct comparison with authentic (+)-pinoresinol.

(+)-1-Hydroxypinoresinol (2)—Colorless crystalline powder, mp 183–185°C, $[\alpha]_D^{15} + 39.0^\circ$ ($c=0.65$ in EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 232 (4.27), 281.0 (3.88). UV $\lambda_{\text{max}}^{\text{EtOH}+\text{NaOH}}$ nm: 253, 293.3. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3540 (OH), 1605, 1510 (arom. C=C). MS: Calcd for $\text{C}_{20}\text{H}_{22}\text{O}_7$, 374.1364. Obsd., 374.1367. CD ($c=1.458 \times 10^{-4}$, ethanol) $[\theta]^{20} \times 10^{-3}$ (nm): +1.03 (277), -2.91 (238), +8.22 (212). ^1H -NMR (in CDCl_3) δ : 3.00–3.23 (1H, m, $\text{C}_5\text{-H}$), 3.87 (6H, s, $2 \times \text{OCH}_3$), 3.65–4.22 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.53 (1H, dd, $J=8$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.80 (1H, s, $\text{C}_2\text{-H}$), 4.85 (1H, d, $J=5$ Hz, $\text{C}_6\text{-H}$), 6.67–7.10 (6H, m, arom. H). The identity of this compound was confirmed by direct comparison with authentic (+)-1-hydroxypinoresinol.

(+)-Fraxiresinol (3)—Amorphous powder, $[\alpha]_D^{25} + 29.3^\circ$ ($c=0.61$ in CHCl_3), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 232.3 (4.10), 279.8 (3.54). UV $\lambda_{\text{max}}^{\text{EtOH}+\text{NaOH}}$ nm: 235.7, 254.9, 284. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3545 (OH), 1615, 1510 (arom. C=C). MS: Calcd for $\text{C}_{21}\text{H}_{24}\text{O}_8$, 404.1470. Obsd., 404.1476. CD ($c=4.862 \times 10^{-4}$, ethanol) $[\theta]^{20} \times 10^{-3}$ (nm): +1.202 (233.0), +0.926 (270.0). ^1H -NMR (in CDCl_3) δ : 2.95–3.22 (1H, m, $\text{C}_5\text{-H}$), 3.85 (9H, s, $3 \times \text{OCH}_3$), 3.70–4.17 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.52 (1H, dd, $J=9$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.80 (1H, s, $\text{C}_2\text{-H}$), 4.85 (1H, d, $J=5$ Hz, $\text{C}_6\text{-H}$), 6.58 (2H, s, arom. $\text{C}_{2',6'}\text{-H}$), 6.73–7.03 (3H, m, arom. $\text{C}_{2'',5'',6''}\text{-H}$).

(+)-Fraxiresinol Dimethyl Ether (3a)—**3** (50 mg) was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using CHCl_3 -AcOEt (2:1) to give **3a** (34.8 mg) as an amorphous powder. $[\alpha]_D^{25} + 13.1^\circ$ ($c=0.58$ in EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 229.6 (4.22), 277.9 (3.58). UV $\lambda_{\text{max}}^{\text{EtOH}+\text{NaOH}}$ nm: 229.6 sh, 277.9. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450 (OH), 1590, 1510 (arom. C=C). MS: Calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8$, 432.1781. Obsd., 432.1765. ^1H -NMR (in CDCl_3) δ : 2.94–3.48 (1H, m, $\text{C}_5\text{-H}$), 3.84 (15H, s, $5 \times \text{OCH}_3$), 3.68–4.16 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.52 (1H, dd, $J=9$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.80 (1H, s, $\text{C}_2\text{-H}$), 4.83 (1H, d, $J=5$ Hz, $\text{C}_6\text{-H}$), 6.58 (2H, s, arom. $\text{C}_{2',6'}\text{-H}$), 6.71–7.03 (3H, m, arom. $\text{C}_{2'',5'',6''}\text{-H}$).

Sodium-Ammonia Reduction of (+)-Fraxiresinol Dimethyl Ether (3a)—**3a** (30 mg) in pure tetrahydrofuran (THF) (2 ml) was added to liquid ammonia, and the stirred solution was treated with sodium (30 mg). After the

disappearance of the blue color, a little water was added to the yellow solution and the ammonia was evaporated off. The residue was treated with H_2O , the mixture was extracted with CHCl_3 , and the extract was concentrated. The residue was purified by preparative TLC using CHCl_3 -AcOEt (1 : 1) to give **3b** as a colorless syrup. $[\alpha]_D^{22} - 15.4^\circ$ ($c = 0.15$ in CHCl_3). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 224.5 (4.16), 278.5 (3.52). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3424 (OH), 1592, 1514 (arom. C=C). MS: Calcd for $\text{C}_{23}\text{H}_{32}\text{O}_8$, 436.2094. Obsd., 436.2085. MS m/z : 436 (M^+ , $\text{C}_{23}\text{H}_{32}\text{O}_8$, 5.4%), 254 ($\text{C}_{13}\text{H}_{18}\text{O}_5$, 4.8%), 237 ($\text{C}_{13}\text{H}_{17}\text{O}_4$, 4.3%), 219 ($\text{C}_{13}\text{H}_{15}\text{O}_3$, 5.4%), 189 ($\text{C}_{12}\text{H}_{13}\text{O}_2$, 7.0%), 182 ($\text{C}_{10}\text{H}_{14}\text{O}_3$, 100%), 181 ($\text{C}_{10}\text{H}_{13}\text{O}_3$, 31.7%), 167 ($\text{C}_9\text{H}_{11}\text{O}_3$, 25.8%), 151 ($\text{C}_9\text{H}_{11}\text{O}_2$, 75.3%), 137 ($\text{C}_8\text{H}_9\text{O}_2$, 25.3%). $^1\text{H-NMR}$ (in CDCl_3) δ : 1.91–2.27 (1H, m, $\text{C}_3\text{-H}$), 2.44–2.71 (2H, m, $\text{C}_4\text{-H}$), 2.90 (2H, s, $\text{C}_1\text{-H}$), 3.35–3.80 (4H, m, $\text{C}_{2a,3a}\text{-H}$), 3.53 (2H, s, $2 \times \text{OH}$, quenched by addition of D_2O), 3.83 (15H, s, $5 \times \text{OCH}_3$), 6.50 (2H, s, arom. $\text{C}_{2',6'}\text{-H}$), 6.63–6.90 (3H, m, arom. $\text{C}_{2'',5'',6''}\text{-H}$).

Reduction of Hydroxythujaplicatin Trimethyl Ether with Lithium Aluminum Hydride—A solution of hydroxythujaplicatin trimethyl ether (136 mg) in THF (5 ml) was added dropwise to a suspension of LiAlH_4 (136 mg) in THF (5 ml). The mixture was stirred for 8 h at room temperature and then poured into ice-cold water. The whole was carefully acidified with 10% H_2SO_4 soln. and extracted with ether. The ether soln. was washed with water and concentrated *in vacuo*. The residue showed a spot of R_f 0.29 on TLC using CHCl_3 -AcOEt (1 : 2). Purification by TLC afforded the triol **3b** as a colorless syrup. All the spectral data of the product were in good agreement with those of the triol **3b**.

(+)-1-Hydroxysyringaresinol (4)—Colorless crystalline powder, mp 95–97°C. $[\alpha]_D^{24.5} + 24.6^\circ$ ($c = 0.12$ in CHCl_3). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 237.0 (4.10), 272.2 (3.40), 281.0 (3.32). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3545 (OH), 1615, 1515 (arom. C=C). MS: Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_9$, 434.1574. Obsd., 434.1562. CD ($c = 5.423 \times 10^{-4}$, ethanol) $[\theta]^{20} \times 10^{-3}$ (nm): +4.05 (262). $^1\text{H-NMR}$ (in CDCl_3) δ : 3.00–3.20 (1H, m, $\text{C}_5\text{-H}$), 3.85 (12H, s, $4 \times \text{OCH}_3$), 3.66–4.20 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.51 (1H, dd, $J = 9$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.78 (1H, s, $\text{C}_2\text{-H}$), 4.81 (1H, d, $J = 5$ Hz, $\text{C}_6\text{-H}$), 6.60 (2H, s, arom. H), 6.62 (2H, s, arom. H).

(+)-1-Hydroxysyringaresinol Triacetate (4a)—**4** (50 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using CHCl_3 -AcOEt (1 : 1) to give **4a** (38.0 mg) as a colorless crystalline powder. mp 81–83°C. $[\alpha]_D^{25} + 12.0^\circ$ ($c = 0.63$ in CHCl_3). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 223.5 (4.23) sh, 274.4 (3.40), 277.8 (3.39). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1758, 1737 (C=O), 1600, 1504 (arom. C=C). MS: Calcd for $\text{C}_{28}\text{H}_{32}\text{O}_{12}$, 560.1890. Obsd., 560.1889. $^1\text{H-NMR}$ (in CDCl_3) δ : 1.70 (3H, s, alcoholic OCOCH_3), 2.30, 2.32 (6H, each s, $2 \times$ phenolic OCOCH_3), 3.17–3.48 (1H, m, $\text{C}_5\text{-H}$), 3.79 (12H, s, $4 \times \text{OCH}_3$), 3.53–4.46 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.52 (1H, dd, $J = 9$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.78 (1H, d, $J = 5$ Hz, $\text{C}_6\text{-H}$), 5.03 (1H, s, $\text{C}_2\text{-H}$), 6.55 (2H, s, arom. H), 6.60 (2H, s, arom. H).

(+)-1-Hydroxysyringaresinol Dimethyl Ether (4b)—**4** (25 mg) was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using CHCl_3 -AcOEt (2 : 1) to give **4b** (14.6 mg) as an amorphous powder. $[\alpha]_D^{22} + 11.9^\circ$ ($c = 0.076$ in CHCl_3). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 226.1 (4.19) sh, 275.7 (3.53). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (OH), 1590, 1512 (arom. C=C). MS m/z : 462 (M^+ , $\text{C}_{24}\text{H}_{30}\text{O}_9$). $^1\text{H-NMR}$ (in CDCl_3) δ : 2.90–3.49 (1H, m, $\text{C}_5\text{-H}$), 3.84 (18H, s, $6 \times \text{OCH}_3$), 3.50–4.40 (3H, m, $\text{C}_{4,8}\text{-H}$), 4.52 (1H, dd, $J = 9$ and 9 Hz, $\text{C}_{4e}\text{-H}$), 4.80 (1H, s, $\text{C}_2\text{-H}$), 4.83 (1H, d, $J = 5$ Hz, $\text{C}_6\text{-H}$), 6.57 (2H, s, arom. H), 6.60 (2H, s, arom. H).

Oxidation of (+)-1-Hydroxysyringaresinol Dimethyl Ether (4b) with Potassium Permanganate—**4b** (10 mg) was dissolved in 10 ml of 1 N NaOH soln. with warming on a water bath. The solution was treated with 2% KMnO_4 soln. in small portions at 35°C (pink end point). The color was discharged with sodium bisulfite solution and the precipitate was filtered off. The filtrate, after being acidified with dil. H_2SO_4 soln., was extracted with ether. The ether solution was evaporated to yield the oxidation product as colorless needles, mp 169–171°C. The identity of this product was confirmed by direct comparison with authentic 3,4,5-trimethoxybenzoic acid.

(-)-Olivil (5)—Colorless needles from EtOH, mp 121–123°C. $[\alpha]_D^{23} - 23.9^\circ$ ($c = 1.29$ in EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 230.4 (4.10), 281.5 (3.72). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1600, 1510 (arom. C=C). MS m/z : 376 (M^+ , $\text{C}_{20}\text{H}_{24}\text{O}_7$). $^1\text{H-NMR}$ (in CD_3OD) δ : 2.10–2.83 (1H, m, $\text{C}_3\text{-H}$), 2.92 (2H, s, $-\text{CH}_2-$), 3.76 (6H, s, $2 \times \text{OCH}_3$), 3.38–3.98 (2H, m, $-\text{CH}_2\text{OH}$), 3.97–4.50 (2H, m, $\text{C}_5\text{-H}$), 6.46–7.15 (6H, m, arom. H). The identity of this product was confirmed by direct comparison with authentic (-)-olivil.

(+)-Cyclo-olivil (6)—Colorless needles from EtOH, mp 168–170°C. $[\alpha]_D^{23} + 51.8^\circ$ ($c = 0.77$ in EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 230.5 (4.03), 284 (3.69). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (OH), 1620, 1520 (arom. C=C). MS m/z : 376 (M^+ , $\text{C}_{20}\text{H}_{24}\text{O}_7$). $^1\text{H-NMR}$ (in CD_3OD) δ : 1.90–2.24 (1H, m, $\text{C}_2\text{-H}$), 2.60 (1H, d, $J = 17$ Hz, $\text{C}_4\text{-H}$), 3.20 (1H, d, $J = 17$ Hz, $\text{C}_4\text{-H}$), 3.73, 3.75 (6H, each s, $2 \times \text{OCH}_3$), 3.40–4.35 (5H, m, $\text{C}_{1,2a,3a}\text{-H}$), 6.15–6.85 (5H, m, arom. H). The identity of this product was confirmed by direct comparison with authentic (+)-cyclo-olivil.

(+)-Pinoresinol- β -D-glucoside (7)—Amorphous powder, mp 107–109°C. $[\alpha]_D^{20} + 8.6^\circ$ ($c = 0.79$ in EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 228.3 (4.14), 280.5 (3.66). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1600, 1510 (arom. C=C). MS m/z : 358, $\text{C}_{20}\text{H}_{22}\text{O}_6$. $^1\text{H-NMR}$ (in CD_3OD) δ : 2.93–3.18 (2H, m, $\text{C}_{1,5}\text{-H}$), 3.80 (6H, s, $2 \times \text{OCH}_3$), 3.58–4.44 (4H, m, $\text{C}_{4,8}\text{-H}$), 6.60–7.21 (6H, m, arom. H). $^{13}\text{C-NMR}$ (in $\text{DMSO}-d_6$) δ : 53.5 (C-1, 5), 84.8 (C-2), 70.9 (C-4, 8), 85.1 (C-6), 135.2 (C-1'), 110.4 (C-2', 2''), 148.9 (C-3', 3''), 145.9 (C-4', 4''), 115.1 (C-5', 5''), 118.1 (C-6'), 132.1 (C-1''), 118.6 (C-6''), 55.6 (OCH_3), 100.1 (Glc-1), 73.1 (Glc-2), 76.9 (Glc-3, 5), 69.6 (Glc-4), 60.6 (Glc-6).

Hydrolysis of (+)-Pinoresinol- β -D-glucoside (7) with Emulsin—**7** (10 mg) was hydrolyzed with emulsin in the usual way to give **1** and D-glucose. The lignan **7** was identified by direct comparison with authentic (+)-pinoresinol- β -

D-glucoside.

(+)-1-Hydroxypinoresinol-4'- β -D-glucoside (8)—Colorless plates, mp 127–129°C. $[\alpha]_D^{23}$ -9.3° ($c=0.42$ in MeOH). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 228.0 (4.11), 279.8 (3.66). UV $\lambda_{\max}^{\text{EtOH}+\text{NaOH}}$ nm: 252, 280, 292 sh. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1600, 1515 (arom. C=C). CD ($c=3.444 \times 10^{-4}$, ethanol) $[\theta]^{20} \times 10^{-3}$ (nm): -0.59 (271), -1.90 (239), $+1.77$ (214). FD-MS m/z : 536 (M^+ , $C_{26}H_{32}O_{12}$). $^1\text{H-NMR}$ (in CD_3OD) δ : 2.96–3.14 (1H, m, $C_5\text{-H}$), 3.80, 3.83 (6H, each s, $2 \times \text{OCH}_3$), 3.47–4.58 (4H, m, $C_{4,8}\text{-H}$), 4.80 (1H, s, $C_2\text{-H}$), 6.60–6.80 (6H, m, arom. H). $^{13}\text{C-NMR}$ (in $\text{DMSO-}d_6$) δ : 91.1 (C-1), 86.8 (C-2), 70.2 (C-4), 60.8 (C-5), 85.4 (C-6), 74.7 (C-8), 131.1 (C-1'), 112.6 (C-2'), 148.3 (C-3'), 146.0 (C-4', 4''), 114.6 (C-5'), 119.7 (C-6'), 132.3 (C-1''), 110.8 (C-2''), 147.5 (C-3''), 115.1 (C-5''), 118.8 (C-6''), 55.6 (OCH_3), 100.4 (Glc-1), 73.2 (Glc-2), 76.8 (Glc-3, 5), 69.7 (Glc-4), 60.8 (Glc-6).

(+)-1-Hydroxypinoresinol-4'- β -D-glucoside Hexaacetate (8a)—**8** (16 mg) was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC using $\text{CHCl}_3\text{--AcOEt}$ (1:1) to give **8a** (15.5 mg) as a colorless syrup. $[\alpha]_D^{20}$ -6.3° ($c=1.8$ in EtOH). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 220 (4.26), 275 (3.75), 279 (3.74). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 1720 (C=O), 1595, 1500 (arom. C=C). MS m/z : 788 (M^+ , $C_{38}H_{44}O_{18}$). $^1\text{H-NMR}$ (in CDCl_3) δ : 1.67 (3H, s, alcoholic OCOCH_3), 2.03, 2.10 (12H, each s, $4 \times$ alcoholic OCOCH_3), 2.33 (3H, s, phenolic OCOCH_3), 3.10–3.55 (1H, m, $C_5\text{-H}$), 3.83, 3.87 (6H, each s, $2 \times \text{OCH}_3$), 4.10–4.55 (4H, m, $C_{4,8}\text{-H}$), 4.82 (1H, d, $J=5$ Hz, $C_6\text{-H}$), 5.08 (1H, s, $C_2\text{-H}$), 6.73–7.27 (6H, m, arom. H).

Hydrolysis of (+)-1-Hydroxypinoresinol-4'- β -D-glucoside (8) with Emulsin—**8** (16 mg) was hydrolyzed with emulsin in the usual way to give **2** and D-glucose.

(+)-1-Hydroxypinoresinol 4''-O-Methyl Ether-4'- β -D-glucoside (8b)—**8** (28.2 mg) was methylated with diazomethane in the usual way. The crude product was purified by preparative TLC using $\text{CHCl}_3\text{--EtOH}$ (4:1) to give **8b** (10 mg) as an amorphous powder. mp 117–120°C. $[\alpha]_D^{23}$ -1.3° ($c=1.0$ in EtOH). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 230 (4.26), 277 (3.76). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1590, 1510 (arom. C=C). Anal. Calcd for $C_{27}H_{34}O_{12} \cdot 1.5\text{H}_2\text{O}$: C, 56.14; H, 6.46. Found: C, 56.23; H, 6.18. $^1\text{H-NMR}$ (in CD_3OD) δ : 2.92–3.10 (1H, m, $C_5\text{-H}$), 3.77, 3.79, 3.83 (9H, each s, $3 \times \text{OCH}_3$), 3.52–4.38 (4H, m, $C_{4,8}\text{-H}$), 4.82 (1H, d, $J=5$ Hz, $C_6\text{-H}$), 4.73 (1H, s, $C_2\text{-H}$), 6.59–7.16 (6H, m, arom. H). $^{13}\text{C-NMR}$ (in $\text{DMSO-}d_6$) δ : 91.2 (C-1), 86.8 (C-2), 70.3 (C-4), 60.8 (C-5), 85.1 (C-6), 74.7 (C-8), 131.0 (C-1'), 112.5 (C-2'), 148.3 (C-3'), 145.9 (C-4'), 114.6 (C-5'), 119.7 (C-6'), 133.9 (C-1''), 110.2 (C-2''), 148.7 (C-3''), 148.3 (C-4''), 111.6 (C-5''), 118.4 (C-6''), 55.5, 55.7 (OCH_3), 100.3 (Glc-1), 73.2 (Glc-2), 76.9 (Glc-3, 5), 69.7 (Glc-4), 60.8 (Glc-6).

Hydrolysis of (+)-1-Hydroxypinoresinol 4''-O-Methyl Ether-4'- β -D-glucoside (8b) with Emulsin—**8b** (25 mg) was hydrolyzed with emulsin in the usual way to give **8c** (12 mg) and D-glucose.

(+)-1-Hydroxypinoresinol 4''-O-Methyl Ether (8c)—Amorphous powder. $[\alpha]_D^{23}$ $+37.9^\circ$ ($c=0.81$ in CHCl_3). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 232.5 (4.21), 280 (3.75). UV $\lambda_{\max}^{\text{EtOH}+\text{NaOH}}$ nm: 235, 253, 285, 295. IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1} : 3540 (OH), 1605, 1590, 1510 (arom. C=C). MS: Calcd for $C_{21}H_{24}O_7$, 388.1520. Obsd., 388.1538. CD ($c=4.727 \times 10^{-4}$, ethanol) $[\theta]^{20} \times 10^{-3}$ (nm): $+1.06$ (278), -0.36 (242.5), $+0.32$ (240), $+0.33$ (228). $^1\text{H-NMR}$ (in CDCl_3) δ : 1.80 (1H, br s, OH, quenched by addition of D_2O), 2.94–3.33 (1H, m, $C_5\text{-H}$), 3.91 (9H, s, $3 \times \text{OCH}_3$), 3.42–4.72 (4H, m, $C_{4,8}\text{-H}$), 4.83 (1H, s, $C_2\text{-H}$), 4.88 (1H, d, $J=5$ Hz, $C_6\text{-H}$), 6.59–7.24 (6H, m, arom. H). The identity of this product was confirmed by direct comparison with authentic (+)-1-hydroxypinoresinol 4''-O-methyl ether.

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