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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

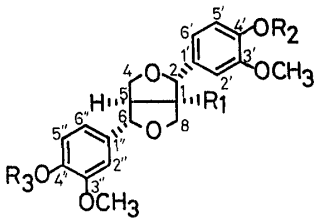
Fraxinus mandshurica RUPR. var. *japonica* MAXIM (Oleaceae) is widely distributed in Hokkaido, the northern part of Japan. In China, the dried bark of *F. mandshurica* is sometimes used as a substitute for the Chinese crude drug "qin pi (Cortex Fraxini, 秦皮)".[1] Further, the dried bark of *F. japonica* 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]

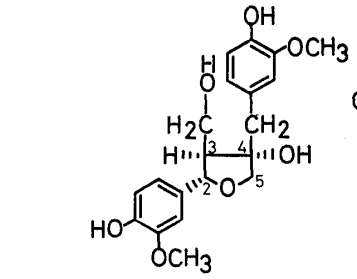
Thus, our interest has been directed to the investigation of the constituents of these *Fraxinus* barks. This paper describes the isolation of three new lignans, **3**, **4** and **8**, along with five known lignans, (+)-pinoresinol (**1**), (+)-1-hydroxypinoresinol (**2**), (−)-olivil (**5**), (+)-cyclo-olivil (**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, $C_{21}H_{24}O_8$, $[\alpha]_D^{25}$ +29.3° (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 (^{1}H-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



1:	$R_1=R_2=R_3=H$		
1a:	$R_1=H$	$R_2=R_3=CH_3$	
2:	$R_1=OH$	$R_2=R_3=H$	
2a:	$R_1=OH$	$R_2=R_3=CH_3$	
7:	$R_1=H$	$R_2=glc$	$R_3=H$
8:	$R_1=OH$	$R_2=glc$	$R_3=H$
8a:	$R_1=OCOCH_3$	$R_2=glc(COCH_3)_4$	$R_3=COCH_3$
8b:	$R_1=OH$	$R_2=glc$	$R_3=CH_3$
8c:	$R_1=OH$	$R_2=H$	$R_3=CH_3$
3:	$R_1=OH$	$R_2=R_3=H$	
3a:	$R_1=OH$	$R_2=CH_3$	$R_3=H$
3c:	$R_1=R_2=R_3=H$		
3d:	$R_1=R_3=H$	$R_2=CH_3$	
4:	$R_1=OH$	$R_2=H$	$R_3=OCH_3$
4a:	$R_1=OCOCH_3$	$R_2=COCH_3$	$R_3=OCH_3$
4b:	$R_1=OH$	$R_2=CH_3$	$R_3=OCH_3$
4c:	$R_1=R_2=H$	$R_3=OCH_3$	
4d:	$R_1=H$	$R_2=CH_3$	$R_3=OCH_3$



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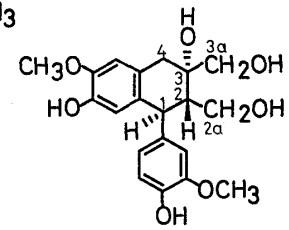

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Chart 1

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° (ethanol). The ^{1}H-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° (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.

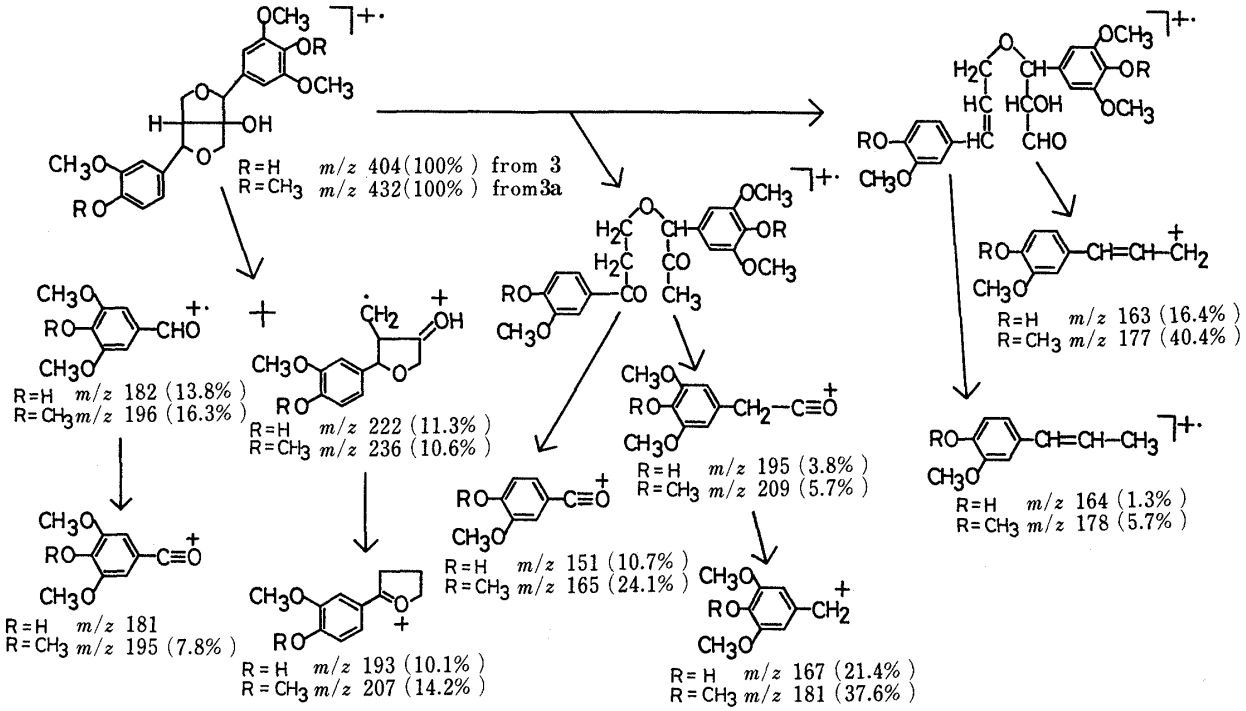
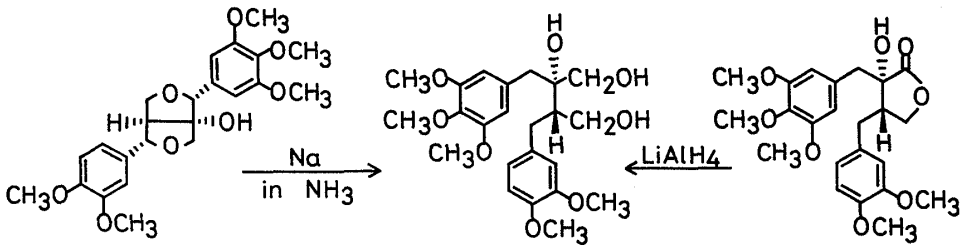


Chart 2. The Mass Fragmentation Patterns of **3** and **3a**



3a **3b** hydroxythujaplicatin trimethyl ether

Chart 3

$[\alpha]_D^{24.5}$ +24.6° (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}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, $C_{28}H_{32}O_{12}$, mp 81—83 °C, $[\alpha]_D^{25}$ +12.0° (chloroform). The ^{1}H-NMR spectrum of **4a** showed the presence of an alcoholic acetoxy group (δ 1.70), two phenolic acetoxy 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, $C_{24}H_{30}O_9$, $[\alpha]_D^{22}$ +11.9° (chloroform). The ^{1}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}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, $C_{20}H_{22}O_6$, 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 (+)-pinoresinol-β-D-glucoside by direct comparison with authentic sample.[7]

The lignan **8** was obtained as colorless plates, $C_{26}H_{32}O_{12}$, 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, $C_{38}H_{44}O_{18}$, $[\alpha]_D^{20}$ −6.3° (ethanol). The ^{1}H-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 ^{13}C-NMR spectrum of **8** also revealed that **8** is a monoglucoside of **2**. Methylation of **8** with diazomethane gave **8b** as an amorphous powder, $C_{27}H_{34}O_{12}\cdot 1.5H_2O$, mp 117—120 °C, $[\alpha]_D^{23}$ −1.3° (ethanol). The enzymatic hydrolysis of **8b** gave **8c** as an amorphous powder, $C_{21}H_{24}O_7$, $[\alpha]_D^{23}$ +37.9° (chloroform) and D-glucose. Compound **8c** was identified as (+)-1-hydroxypinoresinol 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-hydroxypinoresinol-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* (IC_{50} ($\times 10^{-5}$ 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; ^{1}H-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 *Rf* 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 *Rf* 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 *Rf* 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 *Rf* 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° ($c=0.26$ in $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε): 232.0 (4.14), 281.0 (3.75). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3545 (OH), 1610, 1510 (arom. C=C). MS *m/z*: 358 (M^+, $C_{20}H_{22}O_6$). ^{1}H-NMR (in $CDCl_3$) δ: 2.87—3.33 (2H, m, $C_{1,5}$–H), 3.87 (6H, s, $2\times OCH_3$), 3.56—4.46 (4H, m, $C_{4,8}$–H), 4.73 (2H, d, $J=5$ Hz, $C_{2,6}$–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° ($c=0.65$ in EtOH). UV λ_{max}^{EtOH} nm (log ε): 232 (4.27), 281.0 (3.88). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 253, 293.3. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1605, 1510 (arom. C=C). MS: Calcd for $C_{20}H_{22}O_7$, 374.1364. Obsd., 374.1367. CD ($c=1.458\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.03 (277), −2.91 (238), +8.22 (212). ^{1}H-NMR (in $CDCl_3$) δ: 3.00—3.23 (1H, m, C_5–H), 3.87 (6H, s, $2\times OCH_3$), 3.65—4.22 (3H, m, $C_{4,8}$–H), 4.53 (1H, dd, $J=8$ and 9 Hz, C_{4e}–H), 4.80 (1H, s, C_2–H), 4.85 (1H, d, $J=5$ Hz, C_6–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° ($c=0.61$ in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 232.3 (4.10), 279.8 (3.54). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 235.7, 254.9, 284. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3545 (OH), 1615, 1510 (arom. C=C). MS: Calcd for $C_{21}H_{24}O_8$, 404.1470. Obsd., 404.1476. CD ($c=4.862\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.202 (233.0), +0.926 (270.0). ^{1}H-NMR (in $CDCl_3$) δ: 2.95—3.22 (1H, m, C_5–H), 3.85 (9H, s, $3\times OCH_3$), 3.70—4.17 (3H, m, $C_{4,8}$–H), 4.52 (1H, dd, $J=9$ and 9 Hz, C_{4e}–H), 4.80 (1H, s, C_2–H), 4.85 (1H, d, $J=5$ Hz, C_6–H), 6.58 (2H, s, arom. $C_{2',6'}$–H), 6.73—7.03 (3H, m, arom. $C_{2'',5'',6''}$–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° ($c=0.58$ in EtOH). UV λ_{max}^{EtOH} nm (log ε): 229.6 (4.22), 277.9 (3.58). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 229.6 sh, 277.9. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1590, 1510 (arom. C=C). MS: Calcd for $C_{23}H_{28}O_8$, 432.1781. Obsd., 432.1765. ^{1}H-NMR (in $CDCl_3$) δ: 2.94—3.48 (1H, m, C_5–H), 3.84 (15H, s, $5\times OCH_3$), 3.68—4.16 (3H, m, $C_{4,8}$–H), 4.52 (1H, dd, $J=9$ and 9 Hz, C_{4e}–H), 4.80 (1H, s, C_2–H), 4.83 (1H, d, $J=5$ Hz, C_6–H), 6.58 (2H, s, arom. $C_{2',6'}$–H), 6.71—7.03 (3H, m, arom. $C_{2'',5'',6''}$–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° (c= 0.15 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 224.5 (4.16), 278.5 (3.52). IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 1592, 1514 (arom. C=C). MS: Calcd for $C_{23}H_{32}O_8$, 436.2094. Obsd., 436.2085. MS m/z: 436 (M^+, $C_{23}H_{32}O_8$, 5.4%), 254 ($C_{13}H_{18}O_5$, 4.8%), 237 ($C_{13}H_{17}O_4$, 4.3%), 219 ($C_{13}H_{15}O_3$, 5.4%), 189 ($C_{12}H_{13}O_2$, 7.0%), 182 ($C_{10}H_{14}O_3$, 100%), 181 ($C_{10}H_{13}O_3$, 31.7%), 167 ($C_9H_{11}O_3$, 25.8%), 151 ($C_9H_{11}O_2$, 75.3%), 137 ($C_8H_9O_2$, 25.3%). ^{1}H-NMR (in $CDCl_3$) δ: 1.91—2.27 (1H, m, C_3–H), 2.44—2.71 (2H, m, C_4–H), 2.90 (2H, s, C_1–H), 3.35—3.80 (4H, m, $C_{2a,3a}$–H), 3.53 (2H, s, 2×OH, quenched by addition of D_2O), 3.83 (15H, s, 5×OCH_3), 6.50 (2H, s, arom. $C_{2',6'}$–H), 6.63—6.90 (3H, m, arom. $C_{2'',5'',6''}$–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 *Rf* 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° (c=0.12 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 237.0 (4.10), 272.2 (3.40), 281.0 (3.32). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3545 (OH), 1615, 1515 (arom. C=C). MS: Calcd for $C_{22}H_{26}O_9$, 434.1574. Obsd., 434.1562. CD (c=5.423×10^{-4}, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +4.05 (262). ^{1}H-NMR (in $CDCl_3$) δ: 3.00—3.20 (1H, m, C_5–H), 3.85 (12H, s, 4×OCH_3), 3.66—4.20 (3H, m, $C_{4,8}$–H), 4.51 (1H, dd, J=9 and 9 Hz, C_{4e}–H), 4.78 (1H, s, C_2–H), 4.81 (1H, d, J=5 Hz, C_6–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° (c=0.63 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 223.5 (4.23) sh, 274.4 (3.40), 277.8 (3.39). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1758, 1737 (C=O), 1600, 1504 (arom. C=C). MS: Calcd for $C_{28}H_{32}O_{12}$, 560.1890. Obsd., 560.1889. ^{1}H-NMR (in $CDCl_3$) δ: 1.70 (3H, s, alcoholic $OCOCH_3$), 2.30, 2.32 (6H, each s, 2×phenolic $OCOCH_3$), 3.17—3.48 (1H, m, C_5–H), 3.79 (12H, s, 4×OCH_3), 3.53—4.46 (3H, m, $C_{4,8}$–H), 4.52 (1H, dd, J=9 and 9 Hz, C_{4e}–H), 4.78 (1H, d, J=5 Hz, C_6–H), 5.03 (1H, s, C_2–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° (c=0.076 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 226.1 (4.19) sh, 275.7 (3.53).IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1590, 1512 (arom. C=C). MS m/z: 462 (M^+, $C_{24}H_{30}O_9$). ^{1}H-NMR (in $CDCl_3$) δ: 2.90—3.49 (1H, m, C_5–H), 3.84 (18H, s, 6×OCH_3), 3.50—4.40 (3H, m, $C_{4,8}$–H), 4.52 (1H, dd, J=9 and 9 Hz, C_{4e}–H), 4.80 (1H, s, C_2–H), 4.83 (1H, d, J=5 Hz, C_6–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° (c=1.29 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230.4 (4.10), 281.5 (3.72). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1510 (arom. C=C). MS m/z: 376 (M^+, $C_{20}H_{24}O_7$). ^{1}H-NMR (in CD_3OD) δ: 2.10—2.83 (1H, m, C_3–H), 2.92 (2H, s, –C<u>H</u>$_2$–), 3.76 (6H, s, 2×OCH_3), 3.38—3.98 (2H, m, –C<u>H</u>$_2$OH), 3.97—4.50 (2H, m, C_5–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° (c=0.77 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 230.5 (4.03), 284 (3.69). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1620, 1520 (arom. C=C). MS m/z: 376 (M^+, $C_{20}H_{24}O_7$). ^{1}H-NMR (in CD_3OD) δ: 1.90—2.24 (1H, m, C_2–H), 2.60 (1H, d, J=17 Hz, C_4–H), 3.20 (1H, d, J= 17 Hz, C_4–H), 3.73, 3.75 (6H, each s, 2×OCH_3), 3.40—4.35 (5H, m, $C_{1,2a,3a}$–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° (c=0.79 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 228.3 (4.14), 280.5 (3.66). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1510 (arom. C=C). MS m/z: 358, $C_{20}H_{22}O_6$). ^{1}H-NMR (in CD_3OD) δ: 2.93—3.18 (2H, m, $C_{1,5}$–H), 3.80 (6H, s, 2×OCH_3), 3.58—4.44 (4H, m, $C_{4,8}$–H), 6.60—7.21 (6H, m, arom. H). ^{13}C-NMR (in 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 λ_{max}^{EtOH} nm (log ε): 228.0 (4.11), 279.8 (3.66). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 252, 280, 292 sh. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1515 (arom. C=C). CD ($c=3.444\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): −0.59 (271), −1.90 (239), +1.77 (214). FD-MS m/z: 536 (M^+, $C_{26}H_{32}O_{12}$). ^{1}H-NMR (in CD_3OD) δ: 2.96—3.14 (1H, m, C_5–H), 3.80, 3.83 (6H, each s, 2×OCH_3), 3.47—4.58 (4H, m, $C_{4,8}$–H), 4.80 (1H, s, C_2–H), 6.60—6.80 (6H, m, arom. H). ^{13}C-NMR (in 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 $CHCl_3$–AcOEt (1 : 1) to give **8a** (15.5 mg) as a colorless syrup. $[\alpha]_D^{20}$ −6.3° (c=1.8 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 220 (4.26), 275 (3.75), 279 (3.74). IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O), 1595, 1500 (arom. C=C). MS m/z: 788 (M^+, $C_{38}H_{44}O_{18}$). ^{1}H-NMR (in $CDCl_3$) δ: 1.67 (3H, s, alcoholic $OCOCH_3$), 2.03, 2.10 (12H, each s, 4×alcoholic $OCOCH_3$), 2.33 (3H, s, phenolic $OCOCH_3$), 3.10—3.55 (1H, m, C_5–H), 3.83, 3.87 (6H, each s, 2×OCH_3), 4.10—4.55 (4H, m, $C_{4,8}$–H), 4.82 (1H, d, J=5 Hz, C_6–H), 5.08 (1H, s, C_2–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 $CHCl_3$–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 λ_{max}^{EtOH} nm (log ε): 230 (4.26), 277 (3.76). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1590, 1510 (arom. C=C). *Anal.* Calcd for $C_{27}H_{34}O_{12}\cdot1.5H_2O$: C, 56.14; H, 6.46. Found: C, 56.23; H, 6.18. ^{1}H-NMR (in CD_3OD) δ: 2.92—3.10 (1H, m, C_5–H), 3.77, 3.79, 3.83 (9H, each s, 3×OCH_3), 3.52—4.38 (4H, m, $C_{4,8}$–H), 4.82 (1H, d, J=5 Hz, C_6–H), 4.73 (1H, s, C_2–H), 6.59—7.16 (6H, m, arom. H). ^{13}C-NMR (in 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 ° (c=0.81 in $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 232.5 (4.21), 280 (3.75). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 235, 253, 285, 295. IR $\nu_{max}^{CHCl_3}$ 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 ($c=4.727\times10^{-4}$, ethanol) $[\theta]^{20}\times10^{-3}$ (nm): +1.06 (278), −0.36 (242.5), +0.32 (240), +0.33 (228). ^{1}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–H), 3.91 (9H, s, 3×OCH_3), 3.42—4.72 (4H, m, $C_{4,8}$–H), 4.83 (1H, s, C_2–H), 4.88 (1H, d, J=5 Hz, C_6–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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References and Notes

1) Wu Jia-Lin, Shen Jie, and Xie Zong-Wan, *Acta Pharmaceutica Sinica*, **18**, 369 (1983).
2) T. Kariyone and Y. Kimura, "Saishin Wakanyakuyoshokubutsu," Hirokawa Shoten, Tokyo, 1976, p. 105.
3) *a*) M. Terazawa and T. Sasaya, *Research Bulletins of College Experiment Forests, Hokkaido University*, **26-1**, 172 (1968); *b*) M. Terazawa and T. Sasaya, *Mokuzai Gakkaishi*, **16**, 192 (1970).
4) *a*) G. Iida, *Tohoku J. Exp. Med.*, **25**, 455 (1935); *b*) G. Shimada, *Yakugaku Zasshi*, **60**, 508 (1940); *c*) N. Morita and M. Hori, *ibid.*, **73**, 771 (1953).
5) I. Yamagami, Y. Suzuki, and K. Itoh, *Folia Pharmacologica Japonica*, **64**, 714 (1968).
6) H. Kodaira, M. Ishikawa, Y. Komoda, and T. Nakajima, *Chem. Pharm. Bull.*, **31**, 2262 (1983).
7) S. Nishibe, M. Chiba, and S. Hisada, *Yakugaku Zasshi*, **97**, 1134 (1977).
8) H. Tsukamoto, S. Hisada, and S. Nishibe, *Chem. Pharm. Bull.*, **32**, 2730 (1984).
9) H. Fujimoto and T. Higuchi, *Mokuzai Gakkaishi*, **23**, 405 (1977).
10) S. Nishibe, M. Chiba, and S. Hisada, *Yakugaku Zasshi*, **97**, 1366 (1977).
11) E. E. Dickey, *J. Org. Chem.*, **23**, 179 (1958).
12) T. Nikaido, T. Ohmoto, T. Kinoshita, U. Sankawa, S. Nishibe, and S. Hisada, *Chem. Pharm. Bull.*, **29**, 3586 (1981).
13) I. Weinryb, M. Chasin, C. A. Free, D. N. Harris, H. Goldenbery, I. M. Michel, V. S. Paik, M. Phillips, S. Samaniego, and S. M. Hess, *J. Pharm. Sci.*, **61**, 1556 (1972).