PHENOLIC COMPOUNDS IN LIVING TISSUES OF WOODS. VII.*

(+)-Pinoresinol monoglucoside in *Fraxinus mandshurica* Rupr. var. *Japonica* Maxim. (Oleaceae)

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Received January 10, 1986

1. Introduction

In the previous papers, the isolation of some phenolic compounds such as (±)-syringaresinol[19], fraxinol[17,19], and a lignan from the sapwood of yachidamo *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.[19] and coniferin and syringin[22], coumarin glucosides[18], phenylethanol derivatives (secoiridoid glucosides)[25] from the inner bark of the same wood were reported (Fig. 1). During in this course of the investigations of phenolic compounds in living tissues of woods[20~28], two additional phenolic compounds FGa and FG were isolated from the inner bark of yachidamo.

This paper deals with the isolation and structural elucidation of the two phenolic compounds FGa and FG and the seasonal variations of the compound FG in the young shoot of the wood.

2. Results

Two phenolic compounds FGa and FG were isolated from the ethanol extractives of the inner bark of yachidamo (*F. mandshurica*) by silica gel column chromatography using ethyl acetate saturated with water as a developing solvent. The compound FGa came out in an aglycon fraction and purified by repeated silica gel column chromatography using *n*-hexane and ethyl acetate (3 : 1—1 : 1, v/v, gradient). It was obtained as colorless crystals, melting at 122°C with $[\alpha]_D$+91.6° (c=1.0, in MeOH). The compound FG

[J. Fac. Agr. Hokkaido Univ., Vol. 62, Pt. 4. 415–428 1986]

* Series VI; See Ref. 25.

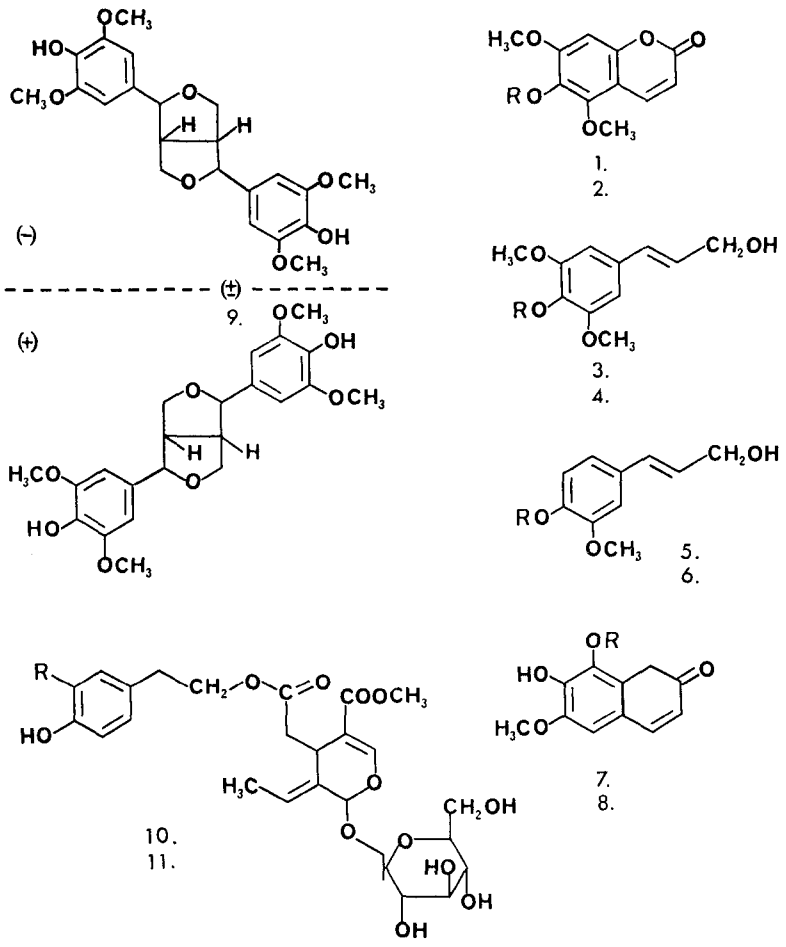

Fig. 1. Phenolic compounds isolated from yachidamo, *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.

Legends: 1.: R=H: fraxinol; 2.: R=Glu: mandshurin; 3.: R=H: sinapyl alcohol; 4.: H=Glu: syringin; 5.: R=H: coniferyl alcohol; 6.: R=H: coniferin; 7.: R=H fraxetin; 8.: R=Glu: fraxin; 9.: (±)-syringaresinol; 10.: R=H: ligustroside; 11.: R=OH: oleuropein.

was obtained from a glycoside fraction as colorless crysyals, melting at 107–109°C with $[\alpha]_D+16°$ (c=0.5, in EtOH). The compound FG yielded an aglycon and glucose by hydrolysis with β-glucosidase. The Rf value and color reaction of the aglycon on thin layer chromatography (TLC) were identical with those of the compound FGa isolated from the aglycon fraction. The results indicated that the compound FG was a glucoside of FGa,

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the compound FGa indicated that it is a lignan with two guaiacyl nuclei and a tetrahydrofurofran ring: six protons of two aromatic methoxyl groups at δ 3.80, two protons of two phenolic hydroxyl groups at δ 7.37, six aromatic protons of two guaiacyl nuclei between δ 6.98–6.65; two protons of two C_α methine groups at δ 4.70, two protons of two C_β methine groups at δ 3.05, and four protons of two C_γ methylene groups at δ 4.1 and 4.05. The data are identical with those of pinoresinol published.[12] It was identified to be (+)-pinoresinol by the comparison of the chromatographic behaviors of an authentic specimen on TLC. Melting point and specific rotation were identical with those of (+)-pinoresinol published.[4]

The ^{1}H-NMR spectrum of FG acetate shows three protons of one aromatic acetoxyl group at δ 2.30, three protons of aromatic methoxyl group at δ 3.84 and 3.83 and the multiplet between δ 6.90–7.11 corresponding to the six aromatic protons of the two guaiacyl nuclei. Two protons of two C_α methine groups are observed at δ 4.80 and 4.76 and two protons of two C_β methine groups are observed between δ 3.10–3.07. Four protons of two C_γ methylene groups are observed at δ 3.94 and 3.93 ($C_{\gamma'}$, double doublets, J=4.5 Hz) and δ 4.25 and 4.30 ($C_{\gamma'}$, multiplets), respectively. In addition to these signals derived from pinoresinol moiety, signals derived from glucosyl residue are observed: twelve protons of four methyl groups of alcoholic acetoxyl groups at δ 2.03 and 2.01, seven protons of five methine groups and one methylene group between δ 3.74–5.30. The data indicate that the compound FG is a pinoresinol monoglucoside.

The highest mass ion at m/z 730 in the mass spectrum (MS) of FG acetate corresponds to the molecular ion (M^+) of pinoresinol monoglucoside pentaacetate ($C_{36}H_{42}O_{16}$, W=730). The fragment ions at m/z 400 and 331 are corresponding to those of pinoresinol monoacetate and the glucosyl residue, respectively. The fragment ion peak at m/z 358 is corresponding to pinoresinol itself. The ionization voltage 20 eV was used for detection of these high mass fragment ions including the molecular ion. However, the intensities of these high mass ions were very weak. In contrast, field desorption (FD) mass spectrum of FG acetate shows the molecular ion clearly at m/z 730 and a fragment ion corresponding to $[M-42]^+$ at m/z 688. FD-MS spectrum of the compound FG shows the molecular ion (M^+) at m/z 520 as pinoresinol monoglucoside ($C_{26}H_{32}O_{11}$, MW=520) and peak at m/z 358 corresponding to $[M+H-163]^+$. The peak at m/z 163 is corresponding to glucosyl residue[9]. These mass spectral data indicate that one of the two phenolic hydroxyl groups of (+)-pinoresinol is blocked by a glucosyl residue.

The ^{13}C-NMR spectral data (^{1}H decoupled (COM)) of the compound FG well support the conclusion derived from the data of ^{1}H-NMR and MS spectra. The peaks at δ 84.91 and 85.20 are those of C_{α} and $C_{\alpha'}$ and peaks at δ 70.97 and 71.06 are corresponding to C_{β} and $C_{\beta'}$, respectively. The peaks at δ 53.60 and 53.75 are corresponding to C_{γ} and C_{γ}'. The peaks at δ 55.77 and 55.67 are two carbons of aromatic methoxyl groups. The peaks of aromatic carbons are observed as follows: 147.57, 148.99 (C_4, $C_{4'}$), 145.90, 145.95 (C_3, $C_{3'}$), 132.26, 135.30 (C_1, $C_{1'}$), 118.18, 118.68 (C_6, $C_{6'}$), 115.20, 115.33 (C_5, $C_{5'}$) and 110.46, 110.65 (C_2, $C_{2'}$). In addition to these peaks derived from pinoresinol moiety, the peaks corresponding to glucose carbons are observed. The peak of C-1 is observed in the lowest field among the carbons of glucosyl residue at δ 100.24 and the peak of C-6 in the highest field at δ 60.75. The other peaks at δ 76.88, 76.86, 73.26 and 69.75 are corresponding to C-3, C-5,

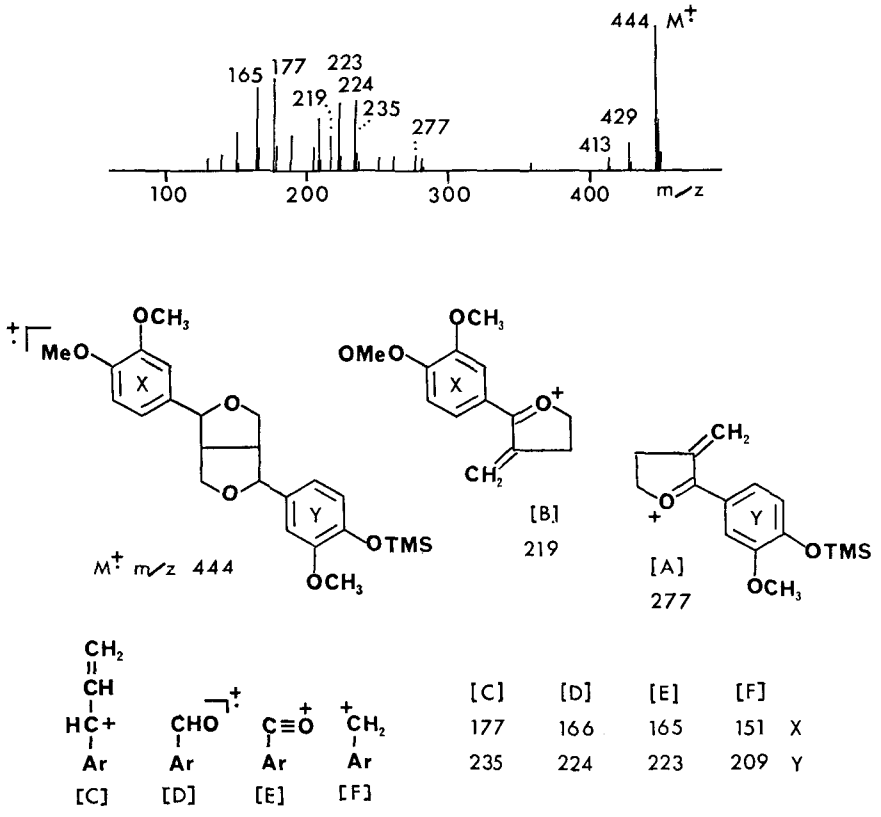

Fig. 2. Mass spectrum of pinoresinol monomethyl ether mono-TMS ether and the main fragment ions.

C-2 and C-4, respectively. The data of the off resonance ^1H decoupling experiment (OFR) of the compound FG show C-H coupling. The information is useful for the assignment of each peak in the COM spectrum.

The compound FG was methylated with diazomethane (CH_2N_2) and the methyl ether derivative was hydrolyzed with 3% sulfuric acid. The aglycon obtained was trimethylsilylated and analyzed by gas-liquid chromatography mass spectroscopy (GC/MS). The highest mass ion at *m/z* 444 is corresponding to pinoresinol monomethyl ether mono-TMS ether ($C_{20}H_{20}O_6T_1$, MW=444, Fig. 2). Fragment ions at *m/z* 277 and 219 are attributed to the fragment [A] and [B], respectively. And the other main fragment ions are corresponding to those containing 3, 4-dimethoxyphenyl nucleus and those containing 3-methoxyl-4-OTMS-phenyl nucleus : [C] : 177 and 235, [D] : 166 and 224, [E] : 165 and 223, and [F] : 151 and 209.

The glucose as the glucosyl residue of the compound FG, was confirmed by the comparison of the data of gas-liquid chromatography (GLC), and paper partition chromatography (PPC) with those an authentic glucose in the same way as described in the previous papers.[22,24,26]

The part of the GLC on the periodical analysis of the ethanol extractives of the inner bark and the xylem of young shoots of yachidamo was shown in the previous paper.[25] Among the many glycosides, (+)-pinoresinol glucoside marked as FG was observed at Rt 87.5 min. The seasonal variations of (+)-pinoresinol monoglucoside in the young shoots of yachidamo are shown in Fig. 3. The amounts of (+)-pinoresinol monoglucoside in the inner bark were between 20-55 mg/g and

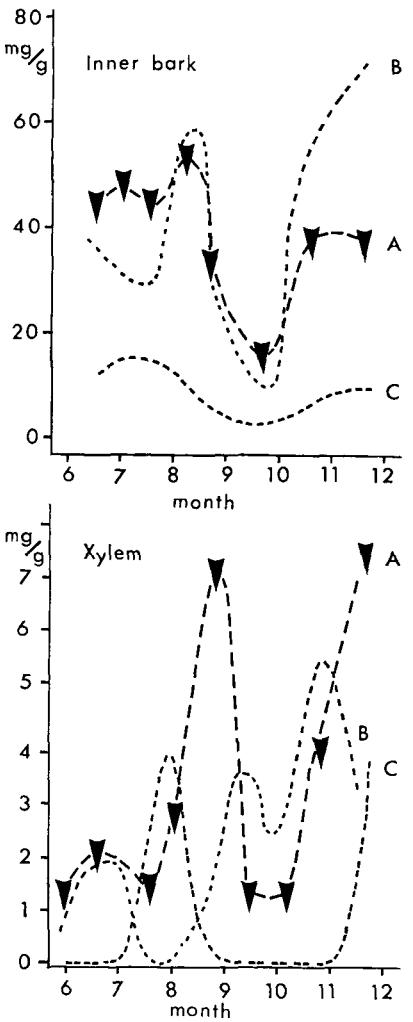

Fig. 3. Seasonal variation of (+)-pinoresinol monoglucoside in ya chidamo, *Fraxinus mandshurica*.

Legends : A : (+)-pinoresinol monoglucoside; B : ligustroside; C : oleuropein.

changed little during the growing season. They reduced after August and increased again toward winter after showing a minimum at the end of September. On the other hand, the variation of (+)-pinoresinol monoglucoside in the xylem was more drastic than that in the inner bark, even its amounts in the xylem were 1/10 of those in the inner bark: (+)-pinoresinol monoglucoside showed a maximum at the end of August and a minimum at the end of September and increased againt toward winter. Judging from its amount in June, the amount of (+)-pinoresinol monoglucoside seems to reduce toward coming spring.

3. Discussion

3.1 Distribution of glycosides of pinoresinols

Phillyrin[10,29] and symplocosin[13] were isolated from *Forsythia* species and from kuroki (*Symplocos lucia*), respectively as the glucoside of pinoresinol derivatives. The former is monoglucoside of (+)-epipinoresinol monomethyl ether and the latter is monoglucoside of (−)-epipinoresinol monomethyl ehter. These glucosides are not optical antipodes because the glucosyl residues are attached to the different aromatic rings each other. Monoglucoside of (+)-epipinoresinol was also isolated from *Forsythia* species.[29] These are all glucosides of epi-type pinoresinols. Dimethyl ehter of (+)-pinoresinol was

TABLE 1. Glycosides of pinoresinol derivatives in plants

Pinoresinols	Plants	Organs	Reference
(−)-Epipinoresinol monomethyl ether monoglucoside =Symplocosin (I)	*Symplocos*	Bark	[13]
(+)-Epipinoresinol monoglucoside (II)	*Forsythia*	Bark	[29]
monomethyl ether monoglucoside =Phillyrin (III)	*Forsythia*	Bark	[10]
(−)-Pinoresinol monoglugoside (IV)	—	—	—
dimethyl ether=Eudesmin (V)*	*Eucalyptus*	Bark	[5]
(+)-Pinoresinol monoglucoside (VI)	*Fraxinus*	Bark	[20, 21]
	Liriodendron	Bark	[6]
	Forsythia	Bark	[2]
	Ligustrum	Bark	[9]
dimethyl ether (VII)	*Magnolia*	Bark	[11]
diglucoside (VIII)	*Liriodendron*	Bark	[6]
(±)-Pinoresinol diglucoside (IX)	*Inomenium*	Bark	[15]

* (+)-Diaeudesmin was isolated from *Piper* as a diastereomer of V and VII [1].

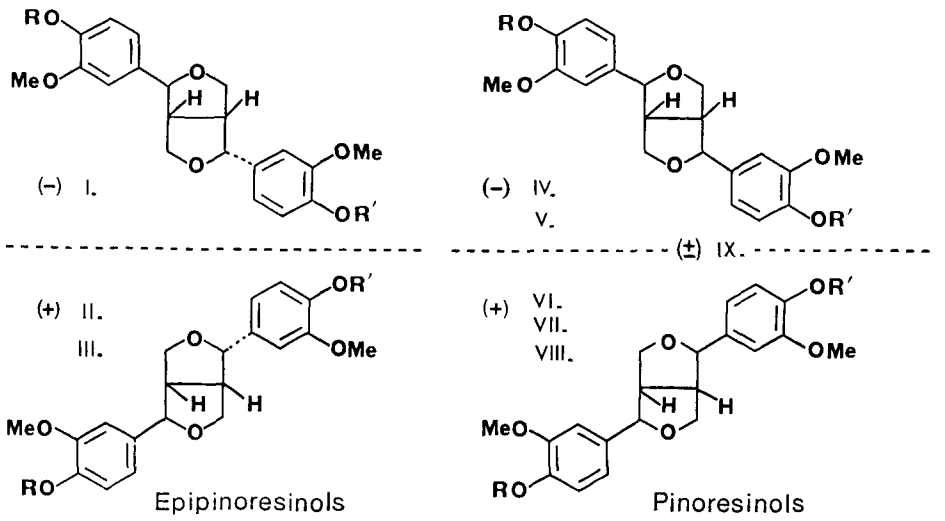

Fig. 4. Pinoresinols in plants.

Legends: I.: R=Me, R′=Glu: (−)-epipinoresinol monomethyl ether monoglucoside (symplocosin); II.: R=H, R′=Glu: (+)-epipinoresinol monoglucoside; III.: R=Glu, R′=Me: (+)-epipinoreinol monomethyl ether monoglucoside (phillyrin); IV.: R=H, R′=Glu: (−)-pinoresinol monoglucoside; V.: R=R′=Me: (−)-pinoresinol dimethyl ether (eudesmin); VI: R=H, R′=Glu: (+)-pinoresinol monoglucoside; VII.: R=R′=Me: (+)-pinoresinol dimethyl ether; VIII.: R=R′=Glu: (+)-pinoresinol diglucoside. IX.: R=R′=Glu: (±)-pinoresinol diglucoside.

found as one of lignans in *Magnolia* (1972).[11] Glycosides of (+)-pinoresinol had not been isolated until TERAZAWA *et al.* isolated (+)-pinoresinol monoglucoside from the inner bark of yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.) and named as pinoresinoside tentatively (in 1972[20] and 1973[21]). FUJIMOTO *et al.*[6] showed later the evidence of the existence of the mono- and diglucosides of (+)-pinoresinol accompanyed by the mono- and diglucosides of (+)-syringaresinol and (+)-medioresinol in the bark of *Liliodendron tulipefera*, although they could not isolate them (1975-1977). KUDO *et al.*[9] isolated a lignan glucoside, which was identified to be (+)-pinoresinol monoglucoside, in addition to many lignan glucosides from the bark of *Ligustrum japonicum* (1976-1980). CHIBA *et al.* also isolated it as one of lignan glucosides from the fruit buds of *Forsythia suspensa* (1978)[2] and reported its ^{13}C-NMR data (1980)[3]. However, no glycosides of (−)-pinoresinol have not been reported so far, although dimethyl ether of (−)-pinoresinol (eudesmin), as a derivative of (−)-pinoresinol, was reported (Table 1). SIH *et al.* isolated diglucoside of (±)-pinoresinol from *Eucommia ulmodes* (1976)[15] (Fig. 4).

3.2 Biogenesis of lignans

TERAZAWA and SASAYA[19] isolated (±)-syringaresinol from the ethanol extractives of the sapwood of yachidamo (1972). The occurrence of the mixture of (+)- and (−)-syringaresinol seemed rather common.[7,8,14] However, no reports on the isolation of (±)-pinoresinol had been published at that time. Based on these facts, they discussed the possibility that (±)-syringaresinol in the xylem might be one of the dimers remained not to be incorporated into lignin macromolecules during lignification in the xylem.

They pointed out the considerable reason why (±)-pinoresinol was difficult to find: coniferyl alcohol and pinoresinol have active sites at 5 and 5′ of the guaiacyl nucleus(ei) to form C–C bonding when they are dehydrogenated by peroxidase in the process of lignification. This nature results in the formation of dehydrogenative polymerization products of higher molecular weight than those of the dimers. Thus there are less chance to isolate (±)-pinoresinol itself in contrast to the frequent encounter to (±)-syringaresinol which dose not have active site at 5, or 5′. They mentioned the result that (±)-syringaresinol was the predominant dimerization products derived from sinapyl alcohol by the catalytic reaction of laccase or peroxidase *in vitro*[19].

In this respect, it is interesting that SIH *et al.* isolated a diglucoside of (±)-pinoresinol from Tu-Chung (*Eucommia ulmodes*) (1976).[15] Namely, the existence of (±)-pinoresinol in the plant suggests the possibility of the existence of the specific enzyme systems to produce them predominantly instead of dehydrogenative polymerization products of coniferyl alcohol produced by catalytic reaction of peroxidase which is involving in lignification process. In the other words, the enzyme systems must have the specific ability to locate the radical at C_β of coniferyl alcohol to lead $C_\beta-C_\beta$ coupling as an initial reaction to form pinoresinols. The finding of the potential existence of the specific enzyme systems is very important because the C_β-C_β coupling is the key point as the initial reaction of the formation of ordinary lignans, even though the enzymes in *Eucommia* lack ability to control the reactions after C_β-C_β coupling to make optically active pinoresinol.

SIH *et al.* used a microorganism *Caldariomlces fumago*, which produce chloroperoxidases, for preparing (±)-pinoresinol and (±)-*cis*-dehydrodiconiferyl alcohol from coniferyl alcohol.[15] The enzyme systems in the microorganism can turn coniferyl alcohol into (±)-pinoresinol and (±)-*cis*-dehydrodiconiferyl alcohol in approximately 1 : 1 ratio, respectively. These enzyme systems also seem to have the specific ability to locate predominantly the radical at C_β of coniferyl alcohol together with the ability to locate the

radical at 5 position of aromatic ring.

The findings of naturally occurring (±)-pinoresinol as its diglucoside in *Eucommia* and of the enzyme systems to form (±)-pinoresinol in *Caldariomlces* suggest that (±)-syringaresinol in hardwoods[7,8,14,19] might be formed by the similar enzyme systems in *Eucommia* rather than be formed by peroxidase which is involving in lignin formation.

STOCKIGT *et al.* studied the biosynthesis of optically active lignans[16] and found that glucoferulic acid, glucoferulic aldehyde and coniferin were good precursors for phillyrin ((+)-epipinoresinol monomethyl ether monoglucoside) but 3, 4-dimethoxycinnamic acid could not be the intermediate. They posturated a biosynthesis way that the first dimerization occurs at C_β carbon atoms of the two coniferyl alcohol units, which are formed by hydrolysis of the glucosidic intermediate coniferin with β-glucosidase. All the reactions including C_β-C_β coupling and ring formation to form (+)-epipinoresinol as the intermediate of phillyrin should be performed by enzyme systems stereospecifically but not through random free radical reaction as seen in polymerization of 4-hydroxycinnamyl alcohols during the formation of optically inactive lignins. Methylation is performed in the final step.

We are now discussing on the enzyme systems which can convert coniferyl alcohol to specific lignan structures. In the next step, we need to find the enzyme systems which can produce optically active lignans *in vitro*. If we could find a pair of enzyme systems which can produce (+)- and (−)-type of a lignan independently, for example (+)-pinoresinol and (−)-pinoresinol, the problem on the natural occurrence of the racemates would be answered somehow.

3.3 Seasonal variation of (+)-pinoresinol monoglucoside (FG) in yachidamo

The seasonal variations of secoiridoid glucosides such as ligustoroside and oleuropein in yachidamo were reported in the previous paper[25]. The increasement of the glucosides toward winter was demonstrated and their decreasement toward spring after showing a maximum in winter was estimated judging from their amounts in June. The patterns of seasonal variations of (+)-pinoresinol monoglucoside (FG) in the inner bark and the xylem demonstrated in this work were similar to those of secoiridoid glucosides reported.[25] On the contrary, the patterns were different from those of hirsutoside in hannoki (*Alnus hirsuta* TURCZ)[23], and platyphylloside in shirakamba (*Betula platyphylla* SUKACHEV var. *japonica* HARA)[24], which decreased toward winter after showing maxima during the growing season.

The physiological significance of these difference in the seasonal variations

of the phenolic glycosides in the living tissues of woods remains uncertain.

4. Experimental

4.1 Isolation of (+)-pinoresinol (FGa) and (+)-pinoresinol monoglucoside (FG)

Two hundred mirigram of the compound FGa ((+)-pinoresinol) and 2.5 g of the compound FG ((+)-pinoresinol monoglucoside) were isolated from 50 g of the ethanol extractives of the inner bark of yachidamo by silica gel column chromatography using ethyl acetate saturated with water in the same way as described in the previous papers.[17~28]

4.2 Physico-chemical properties of (+)-pinoresinol (FGa) and (+)-pinoresinol monoglucoside (FG)

The conditions for UV, IR, MS and NMR spectroscopies are same as described in the previous papers.[24,26,28] The part of NMR (^{1}H-NMR, ^{13}C-NMR) and FD-MS spectroscopies were conducted by a JNM FX-200 FT NMR Spectrometer and by a JOEL JMS-01SG-2 Mass Spectrometer, respectively. $[\alpha]_D$ was measured by a JASCO DIP-360.

4.2.1 (+)-Pinoresinol (FGa): Mp 122°C. $[\alpha]_D$+96.8° (c=1.0, in MeOH). TLC (SG-III): Rf 0.6, pinkish orange with diazotized sulfanilic acid (DSA). *Anal.* Calcd. for $C_{20}H_{22}O_6$: C, 67.02; H, 6.19. Found: C, 67.11; H, 6.06. ^{1}H-NMR (10% in d_6-acetone) δ (ppm): 7.37 (2 H, broad *s*, 2 Ar-OH), 6.98-6.65 (6 H *m*, two aromatic nuclei), 4.70 (2 H, *d*, *J*=4.5 Hz, two C_α methine protons), 4.07-4.12 (2 H, *m*, C_γ methylene), 4.05 (2 H, *m*, $C_{\gamma'}$ methylene), 3.80 (6 H, *s*, two Ar-OMe), and 3.05 (2 H, *m*, two C methine protons).

4.2.2 (+)-Pinoresinol di-TMS ether: (+)-Pinoresinol (FGa) was treated with TMS reagent and the reactant was subjected to GC-MS. MS (20 eV) *m/z*: 502 (M^+), 487 (M-15), 471 (M-31), 430, 306, 294, 277, 235, 223, 209, and 131.

4.2.3 (+)-Pinoresinol dimethyl ether: (+)-Pinoresinol (FGa) was treated with CH_2N_2 and the reactant was subjected to GC-MS. MS (20 eV) *m/z*: 386 (M^+), 371 (M-15), 355 (M-31), 220, 219, 193, 177, 166, 165, and 151.

4.2.4 (+)-Pinoresinol monomethyl ether mono-TMS ether: (+)-Pinoresinol monoglucoside (FG) was dissolved in small amount of ethanol and added the excess amount of CH_2N_2 ether solution and the reaction mixture was evaporated to dryness. The methyl ether was hydrolyzed by a 3% H_2SO_4 solution with refluxing for 30 min., and the cooled hydrolyzate was extracted with ether. The ether soluble part was concentrated to dryness and treated with TMS reagent, then the reactant was subjected to GC-MS. MS (20 eV) *m/z*: 444 (M^+), 429 (M-15), 423 (M-31), 430, 306, 294, 277[A],

262, 235[C], 224[D], 223[E], 219[B], 209[F], 177[C], 166[D], 165[E], and 151[F] (Fig. 2).

4. 2. 5 (+)-Pinoresinol monoglucoside (FG): Mp 106–107°C. $[\alpha]_D^{20}$+16° (c=0.5, in EtOH). UV λ_{max}^{EtOH}. nm (log ε): 282 (3.89). TLC (AEAW): Rf 0.4, pinkish orange with DSA. *Anal.* Calcd. for $C_{26}H_{32}O_{11}$: C, 59.99; H, 6.20. Found C, 59.90; H, 6.22. FD-MS (JOEL JMS-10SG-2, EC 15-16 MA) *m/z* (relative intensities %): 522 (10.2, M+2), 521 (32.1, M+1), 520 (100.0, M^+), 358 (7.87, M+H-163(glu)) 248 (7.7), 179 (7.3). ^{13}C-NMR (2% d_6-DMSO, JNM FX-200 FT NMR) δ (ppm): COM: 53.60, 53.75 (C_γ, $C_{\gamma'}$), 84.91 (C_α), 85.20 ($C_{\alpha'}$), 70.97, 71.06 (C_β, $C_{\beta'}$), 55.77, 55.67 (Ar-OCH_3×2), 132.26 (C_1), 135.30 ($C_{1'}$), 110.46, 110.65 (C_2, $C_{2'}$), 145.90, 145.95 (C_3, $C_{3'}$), 147.57, 148.99 (C_4, $C_{4'}$), 115.20, 115.33 (C_5, $C_{5'}$), 118.18, 118.68 (C_6, $C_{6'}$), 55.67, 55.77 (Ar-OCH_3 ×2), 100.24 (G-C_1), 73.26 (G-C_2), 76.88 (G-C_3), 69.75 (G-C_4), 76.86 (G-C_5), 60.75 (G-C_6).

4. 2. 6 (+)-Pinoresinol monoglucoside pentaacetate: (+)-pinoresinol monoglucoside (FG) was treated with pyridine and acetic anhydride at 55°C for 8 hours and colorless crystals, melting at 109–110°C, were obtained from aqueous ethanol. *Anal.* Calcd. for $C_{36}H_{42}O_{16}$: C, 59.14; H, 5.75. Found: C, 58.56, H, 5.75. ^{1}H-NMR (2% in $CDCl_3$, JNM FX-200 FT NMR) δ (ppm): 3.843, 3.833 (6 H, two *s.*, Ar-OCH_3), 2.308 (3 H, *s.*, Ar-$OCOCH_3$), 6.897–7.108 (6 H, *m.*, Ar-H, two quaiacyl nuclei), 4.801, 4.756 (2 H, two *d.*, J=5 Hz, C_α, $C_{\alpha'}$ methines), 3.104–3.065 (2 H, *m.*, C_β, $C_{\beta'}$ methines), 3.942, 3.930 (2 H, two *d.*, J=4.5 Hz, C_γ methylene), 4.247–4.312 (2 H, *m.*, $C_{\gamma'}$ methylene), 2.034, 2.012 (12 H, two *s.*, four alc-$OCOCH_3$), 3.739–4.312 (3 H, *m.*, glu resid.), 4.928–5.295 (4 H, *m.*, glu resid.). MS (20 eV) *m/z*: 730 (M^+), 688 (M−42), 672, 658, 642, 629, 611, 689, 569, 537, 521, 509, 495, 491, 476, 443, 425, 400, 369, 358, (pinoresinol) 341, 331 (glu resid.), 289, 262, 261, 229, 221, 205, 197, 189, 187, 175, 169, 161, 150, 141. FD-MS (JOEL JMS-01SG-2) *m/z* (relative intensities %): 732 (16.60, M+2), 731 (45.52, M+1), 730 (100.0, M^+), 688 (11.0, M−42) 399 (7.2), 331 (10.2, glu resid.).

4. 2. 7 Glucose as glucosyl residue of the compound FG: The hydrolyzates obtained by hydrolysis of the compound FG with β-glucosidase and by hydrolysis of FG monomethyl ether with 3% sulfuric acid solution were neutralized with $BaCO_3$ and filtrated through Toyo Roshi No. 5C and then evaporated the filtrates to dryness. The parts of them were treated with TMS reagent and subjected to GLC (OV-1). The parts of them were also subjected to PPC (BAW). The Rt and Rf obtained were the same as those of the authentic glucose.[24]

4. 2. 8 Conditions of chromatographies: GLC, TLC and PPC were

the same as described in the previous papers[22,24,26]. GLC conditions for the periodical analysis of FG were the same as described in the previous paper.[25]

Acknowledgement

The author express his appreciation to Professor A. SAKAKIBARA of Hokkaido University for the gift of the authentic (+)-pinoresinol and his helpful advice during this work, to Professor M. MIYAKE of Obihiro University of Agriculture and Veterinary Medicine for his interest and advice during this work and to Mr. M. SASAKI for his experimental assistance. The author express his gratitude to Professor T. KAYAMA of Hokkaido University for his critical reading of this manuscript.

Summary

The compounds FGa and FG, which were isolated from the ethanol extractives of the inner bark of yachidamo *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM., were identified to be (+)-pinoresinol and (+)-pinoresinol monoglucoside, respectively.

Biogenesis of lignins with and without optical activity was discussed.

The seasonal variation of the compound FG in the xylem and the inner bark of the same wood showed two maxima during a year: the growing season and winter. The patterns were similar to those of secoiridoid glycosides in the same wood but different from those of diarylheptanoid glycosides in the woods of Betulaceae.

Literature Cited

1. ATAL, C. K., DHAR, K. L. and PELTER, A.: Isolation and Structure Determination of (+)-Diaeudesmin, the First Naturally Occurring Diaxially Substituted 3, 7-Diaxabicyclo [3, 3, 0] octane Lignan, *J. Chem. Soc.*, 2228-2230. 1967
2. CHIBA, M., HISADA, S. and NISHIBE, S.: Studies on the Chinese Crude Drug "Forsythia Fructus" (III). On the costituents of fruits of *Forsythia viridissima* and *F. guspensa*, *Shyoyakugaku Zasshi* (in Japanese), **32**: 194-197. 1978
3. CHIBA, M., HISADA, S., NISHIBE, S. and THIEME, H.: ^{13}C-NMR Analysis of Symplocosin and (+)-Epipinoresinol Glucoside, *Phytochemistry*, **19**: 335-336. 1980
4. "Dictionary of Organic Compounds", p. 1163, Maruzen Co. Ltd., Tokyo, 1963
5. ERDTMAN, H.: Die Konstitution der Harzphenole und ihre biogenetischen Zusammenhange. II. Über Pinoresinol und seine Bezeihung zu Eudesmin, *Liebigs Ann. Chem.* **516**: 162-169. 1935
6. FUJIMOTO, H., and HIGUCHI, T.: Biosynthesis of Liriodendrin by *Liriodendron turipefera*, *Wood Research*, **62**: 1-10. 1977
7. ISHII, H., OHIDA, O. and HAGINIWA, J.: Studies on the Alkaloids of Rutaceous

Plants. XIX. The Chemical Constituents of *Xanthoxylum inerme* KOIDZ. Isolation of the Chemical Constituents from Bark and Wood, *Yakugaku Zasshi* (in Japanese), **92**: 118–138. 1963

8. ISHII, H., HOSOYA, K., ISHIKAWA, T. and HAGIWARA, J.: Studies on the Chemical Constituents of Rutaceous Plants. XX. The Chemical Constituents of *Xanthoxylum ornottianum* MAXIM. (I). Isolation of the Chemical Constituents of the Xylem of Roots, *ibid.* (in Japanese), **94**: 309–321. 1974
9. KUDO, K., NOHARA, T., KOMORI, T., KAWASAKI, T. and SCHLTOEN, H.-R.: Lignan Glucoside from the Bark of *Ligustrum aponium*, *Plant medica*, **40**: 250–261. 1950
10. KAKU, T., RI, H.: Syntheses von Eudesmin und Pinoresinoldimethyläthers aus l-Asarinin und d-Sesamin, *J. Pharm. Soc Jpn*, **39**: 289–296. 1937
11. KAKISAWA, H., CHEN, Y. P. and HSU, H. Y.: Lignans in Flower Buds of *Magnolia fargasi*, *Phytochemistry*, **11**: 2289–2293. 1972
12. LUDWIG, C. H., NIST, B. J. and MCCARTHY, J. L.: Lignins. XII. The high resolution nuclear magnetic resonance spectroscopy of protons in compounds related to lignin, *J. Amer. Chem. Soc.*, **86**: 1186–1202. 1964
13. OGIYAMA, K. and KONDO, T.: Studies on the Chemical Constituents of Bark of *Symplocos lucida* SIEB. et ZUCC. VI., *Mokuzai Gakkaishi*, **11**: 65–69. 1965
14. SASAKI, Y., MATOBA, K.: Examinations on the Minor Components of *Inomenium acutum* REHD. et WILS. Menispermaceae, *Yakugaku Zasshi* (in Japanese), **87**: 284–286. 1968
15. SIH, C. J., RAVIKUMAR, P. R., HUANG, Fu-C., BUCKNER, C. and WHITLOCK, G.: Isolation and Synthesis of Pinoresinol Diglucoside, A Major Antihypertensive Principle of Tu-Chung (*Eucommia ulmoides* OLIVER), *J. Amer. Chem. Soc.*, **98**: 5412–5413. 1976
16. STOCKIGT, J. and KLISCHIES, M.: Biosynthesis of Lignins. Part. 1. Biosynthesis of arctiin (3) and phyllyrin (5), *Holzforschung*, **31**: 41–44. 1977
17. TERAZAWA, M. and SASAYA, T.: Studies on the Extractives of Yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.). Report 1. The coumarin derivatives and the other compounds of the bark, *Res. Bull. Coll. Exper Forests, Coll. Agric., Hokkaido Univ.*, (in Japanese), **26**: 171–202. 1968
18. TERAZAWA, M. and SASAYA, T.: Studies on the Extractives of Yachidamo *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM. II. Glycosides in bark, *Mokuzai Gakkaishi*, **16**: 192–199. 1970
19. TERAZAWA, M. and SASAYA, T.: Extractives of Yachidamo *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM. III. Extractives of woods. Phenolic compounds in sapwood, *ibid.*, **17**: 167–173. 1971
20. TERAZAWA, H., OKUYAMA, H. and MIYAKE, M.: Phenolic Compounds in Living Tissues of Woods, *Abstracts of The 24th Ann. Meet Jpn. Wood Res. Soc.* (in Japanese), p. 202. 1972
21. TERAZAWA, M., OKUYAMA, H. and MIYAKE, M: Phenolic Compounds in Living Tissue of Woods. Phenolic glycosides in the inner bark of yachidamo *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM., *Proc. Hokkaido Br. Jpn. Wood Res.*

Soc., (in Japanese) **5**: 32–36. 1973

22. TERAZAWA, M., OKUYAMA, H. and MIYAKE, M.: Isolation of Coniferin and Syringin from the Cambial Tissues and Inner Bark of some Angiosperm Woods, *Mokuzai Gakkaishi*, **30**: 409–412. 1984
23. TERAZAWA, M., OKUYAMA, H. and MIYAKE, M.: Phenolic Compounds in Living Tissues of Woods. IV. Hirsutoside from the green bark of keyamahannoki (*Alnus hirsuta*) [Betulaceae] and its seasonal variations in the living tissues of hannoki (*A. japonica*), *ibid.*, **30**: 391–403. 1984
24. TERAZAWA, M., KOGA, T., OKUYAMA, H. and MIYAKE, M.: Phenolic Compounds in Living Tissuesof Woods. III. Platyphylloside, a new diarylheptanoid glucoside from the inner bark of shirakamba (*Betula platyphylla* SUKACHEV. var. *japonica* HARA) [Betulaceae], *ibid.*, **30**: 587–600. 1984
25. TERAZAWA, M.: Phenolic Compounds in Living Tissues of Woods. VI. Ligustroside and oleuropein in the inner bark of *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM. and their seasonal variations in the wood., *Res. Bull. Coll. Exp. Forests, Coll. Agric, Hokkaido Univ.*, **26**: 171–202. 1986
26. TERAZAWA, M., OKUYMAA, H. and MIYAKE, M.: Phenolic Compounds in Living Tissues of Woods. I. Phenolic β-glucosides of 4-hydroxycinnamyl alcohol derivatives in the cambial sap of woods., *Mokuzai Gakkaishi*, **30**: 322–328. 1984
27. TERAZAWA, M. and MIYAKE, M.: Phenolic Compounds in Living Tissues of Woods. II. Seasonal variations of phenolic glycosides in the cambial sap of woods., *ibid.*, **30**: 329–334. 1984
28. TERAZAWA, M., OKUYAMA, M. and MIYAKE, M.: Phenolic Compounds in Living Tissues of Woods. V. Reddish orange staining in keyamahannoki (*Alnus hirsuta*) and hannoki (*A. japonica*) [Betulaceae] caused by the interaction of hirsutoside and catecohol oxidase after cutting the woods., *ibid.*, **30**: 601–607. 1984
29. THIEME, H. and WINKLER, H. J.: Über das Vorkommen von Lignanglucosiden in der Gattung *Forsythia* 1. Mitteilung: Isolierung von Phillyrin und Arctiin, *Phermazie*, **23**: 402–403. 1968