Phenolic Compounds in Living Tissues of Woods. VI.†

Ligustroside and oleuropein in *Fraxinus mandshurica* RUPR. var. *japonica* MAXIM. and *Syringa vulgaris* L. and their seasonal variation in them*1

By

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樹木の生活組織のフェノール成分 (第6報)†

ヤチダモおよびムラサキハシドイ中の ligurstroside および oleuropein の同定とそれらの季節変化*1

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Abstract

Two glycosides FA and FI, which were found in the inner bark of yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.), were identified to be ligustroside (**1**) and oleuropein (**5**), respectively. They were also isolated from the inner bark of murasakihashidoi (*Syringa vulgris* L.).

The glycoside Sy-X, which had been found in cambial sap of murasakihashidoi, was also identified to be oleuropein (**5**). It was concluded that there is no relationship between Sy-X and lignin formation even though the seasonal variation of Sy-X in the cambial sap is so drastic as that of coniferin in the cambial sap of karamatsu (*Larix leptolepis* GORD.).

Their seasonal variations in the inner bark and the xylem of the young shoots of yachidamo were investigated by GLC. The amount of ligustroside (**1**) in the inner bark of the young shoots of the wood changed in the range of 25～80 mg/g during the growing season. It increased toward winter after defoliation. On the contrary, the amounts of oleuropein (**5**) in the inner bark were about 2/5～1/3 of those of ligustroside (**1**) and showed a slight reduction during September and October.

Biosynthesis, chemotaxonomic significance, physiological roles of secoiridoid glucosides were discussed.

Keywords: ligustroside, oleuropein, secoiridoid glucosides, phenylethanols, *Fraxinus*, *Syringa*, chemotaxonomy

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CONTENTS

1. INTRODUCTION

Extractives of yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.) have been investigated and some glycosides of coumarins,[1] lignans,[2] and pehnylethanols[3,4] were isolated. However, several glycosides remain unknown. The isolation and identification of the unknown glycosides FA and FI in the inner bark of yachidamo are of primary importance for the author to discuss chemotaxonomy of the family Oleaceae because these unknown glycosides were also detected by TLC and GLC in the extractives of murasakihashidoi (*Syringa vulgaris* L.). The information on the seasonal variations of the glycosides enables the author to discuss the physiological significance of these glucosides in the wood.

The author has been interested in the compound Sy-X since he found it in the cambial sap of murasakihashidoi as one of the glycosides which showed drastic change during the growing season like coniferin in the cambial sap of karamatsu (*Larix leptolepis* GORD.).[5] Chemical structure of the glycoside Sy-X and its relationship to the lignin formation in murasakihashidoi are discussed.

2. RESULTS

2.1 Identification of the glycoside FA to be ligustroside

The glycoside designated tentatively as FA was isolated from the inner bark of yachidamo as amorphous powder but pure on thin layer chromatography (TLC) and gas liquid chromatography (GLC). Hydrolysis of FA with 3% sulfuric acid gave two aglycons and glucose as the hydrolyzates. The ether soluble part of the hydrolyzates was separated with silica gel column chromatographies. The algycon was identified to be tyrosol, 2-(4′-hydroxyphenyl)ethanol (**9**), which had been isolated as the aglycon of salidroside (**10**) from the the same wood[3,4] and from the inner bark of shirakamba (*Betula platyphylla* var. *japonica* HARA).[3,4] Mild alkaline hydrolysis of FA yielded two compounds, tyrosol (**9**)[6] and an acid glucoside (**22**). This implied that the original compound FA was an ester. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the acid glucoside (as methyl ester acetate **21**, Fig. 1), the signals of the protons of CH_3-CH= exist at δ 1.66 (3 H, *d. d.* J=8 and 2 Hz) and that of CH_3-C*H*= at δ 6.05 (1 H, *quar.*, J=8 Hz). A pair of doublets at δ 2.38 (1 H, J=10, 16 Hz) and 2.75 (1 H, J=5, 18 Hz) and a doublet at δ 3.98

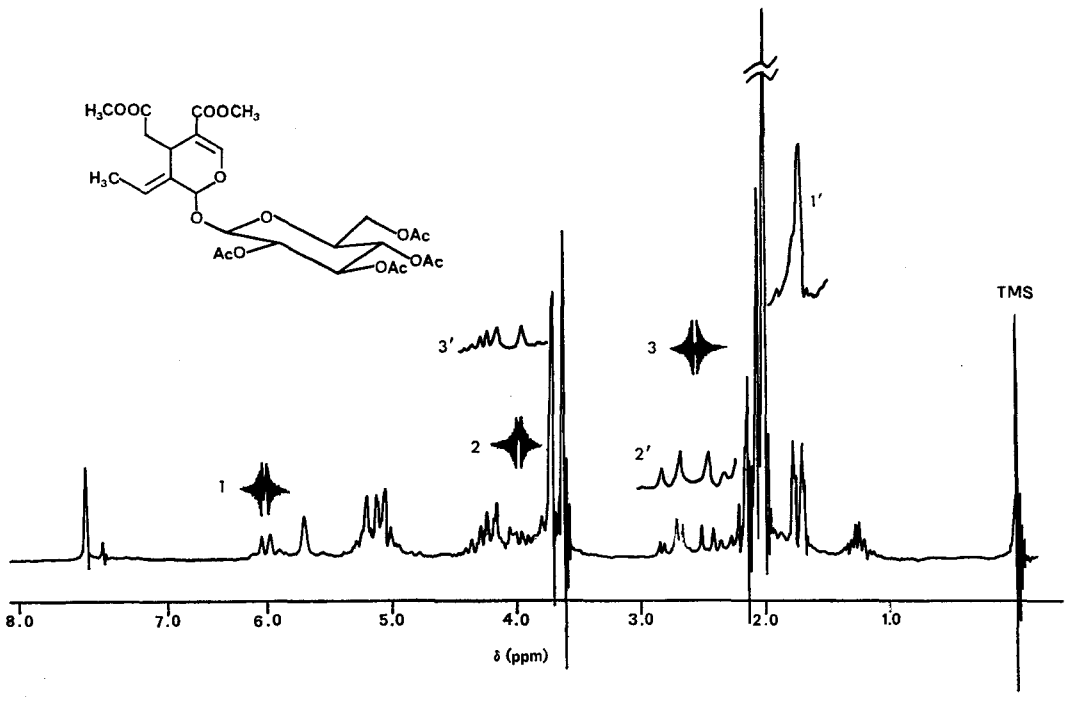


Fig. 1. ^{1}H-NMR spectrum of oleoside dimethyl ester tetraacetate (**21**) derived from oleoside (**22**) which was obtained from both ligustroside (**1**) and oleuropein (**5**) as the mild alkaline hydrolyzate.

Legends: 1—1′, 2—2′, 3—3′: spin decouplings (at 1, 2, and 3) and resulting spectral changes (1′, 2′, 3′).

(1 H, J=5, 10 Hz) indicate the presence of the group of -OOC-CH_AH_B-CH_X-, showing ABX system. The decoupling treatments confirmed this system (Fig. 1). These spectral data show that the glucoside is the secoiridoid glucoside, oleoside (**22**) (as dimethyl ester tetraacetate **21**). Its infra red (IR) absorption spectrum was identical with that of the authentic specimen given by T. Tokoroyama. Then the glucoside FA was supposed to be ligustroside (**1**), the ester of tyrosol (**9**) and oleoside-7-methyl ester (**23**). It was finally identified by the comparison of ^{1}H-NMR spectral data of its tetraacetate (**2**) with those published[7] and with the spectrum of the authentic specimen given by T. Tokoroyama. The mass spectral data of FA acetate (**2**) were identical with those of ligustroside pentaacetate (**2**) published recently.[8] Fig. 2-[A] shows the mass spectrum of FA penta-TMS (trimethylsilyl) ether (**3**). No mass spectral data of ligustroside penta-TMS ether (**3**) have been reported so far. The molecular ion (M^+) of the compound **3** is missing but the highest mass ion peak at m/z 869 is corresponding to the fragment ion of M^+ -15. The fragment ion at m/z 417 is corresponding to the aglycon ester moiety which is formed by sprit of glucosyl residue from the molecular ion. The fragment ions

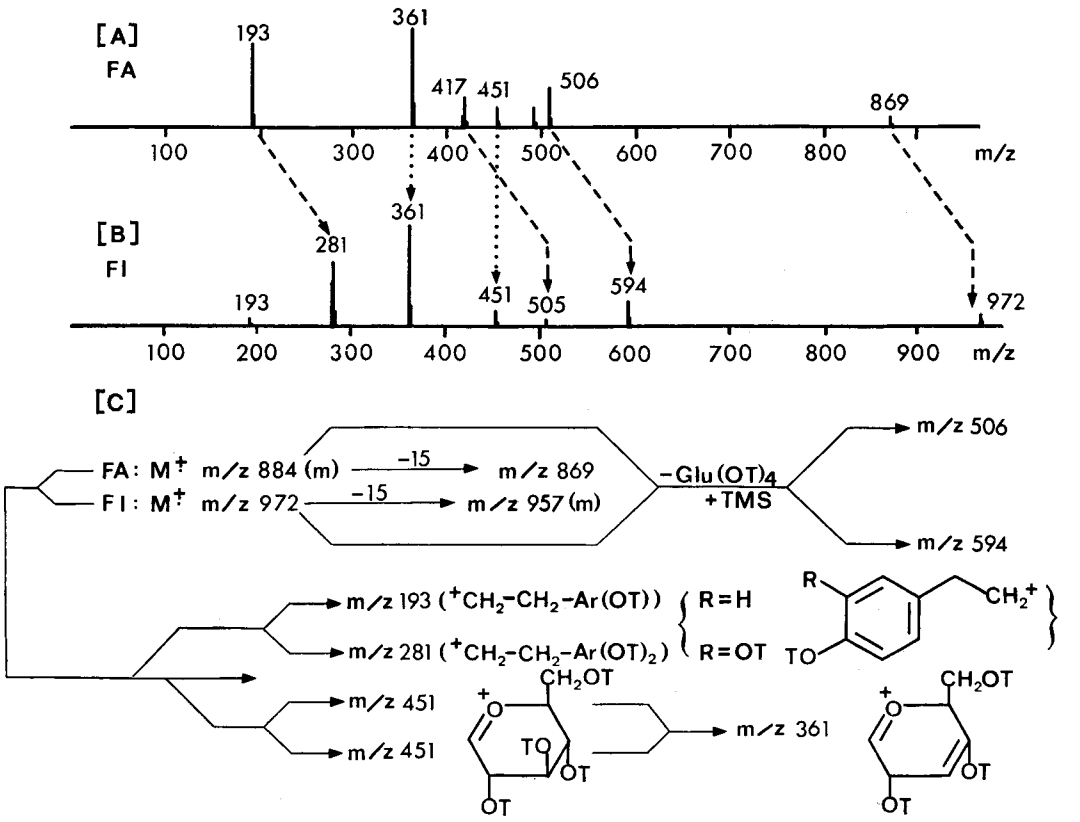


Fig. 2. Mass spectra of liugstroside pentatrimethylsilyl ether (3, FA) [A] and oleuropein hexatrimethylsilyl ether (7, FI) [B], and their mass fragmentations [C].

Legend: (m): missing.

at m/z 451 and 361 are characteristic ones of the trimethylsilyl ether of glucosyl residue. The fragment ion at m/z 193 is corresponding to 4-hydroxyphenylethyl moiety (Fig. 2-[C]).

2.2 Identification of the glycoside FI to be oleuropein

The glycoside designated tentatively as FI was isolated from the inner bark of yachidamo as amorphous powder but pure on TLC and GLC. ^{1}H-NMR spectral data of FI acetate were similar to those of FA acetate, ligustroside pentaacetate (**2**) except the signals in the regions of aromatic and phenolic acetoxyl protons. Mild alkaline hydrolysis of FI yielded 2-(3′,4′-dihydroxyphenyl)ethanol (**11**) as the aglycon and oleoside (**22**), which was identified by the comparison with the oleoside dimethyl ester tetraacetate (**21**) derived from FA. The data indicated the compound FI is the ester of 2-(3′,4′-dihydroxyphenyl)ethanol (**11**) and oleoside 7-methylester (**23**), which is known as oleuropein (**5**). It was finally identified by the comparison of the spectral data of FI and its derivatives with those of oleuropein reported.[9] The chromatographic behaviors of FI on TLC and GLC were identical with those of the authentic

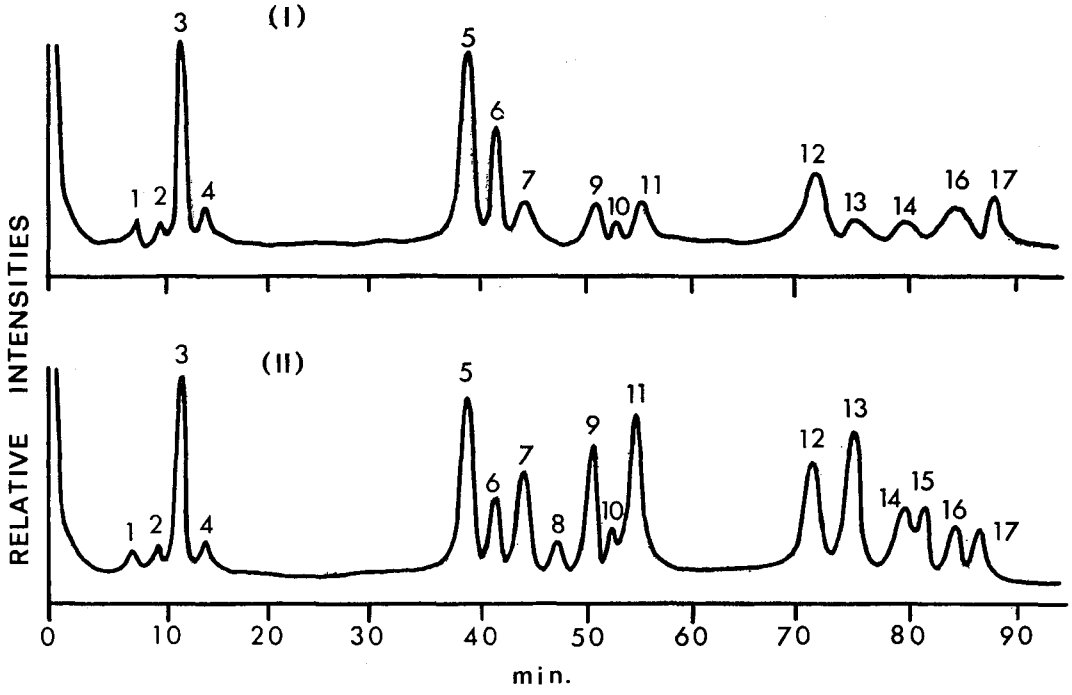


Fig. 3. GLC of the methanol extractives from the xylem and the inner bark of the young shoots of yachidamo (as trimethylsilyl ether derivatives).

Legends: (I): the xylem, (II): the inner bark, 1: fructose, 2 and 4: glucose, 3: mannitol, 5: sucrose, 6: salidroside (FH), 7: fraxidroside (FN), 8: unknown (UK), 9: fraxin (FE), 10: isomandshurin (FNU), 11: mandshurin (FF), 12: ligustroside (FA), 13: oleuropein (FI), 14: fraxiestoside (FQ), 15: UK, 16: pinoresinoside (FG), 17: UK.

specimen given by H. INOUYE. Mass spectral data of FI acetate were identical with those of oleuropein hexaacetate (**6**) published.[8] Fig. 2-[B] shows the mass spectrum of FI TMS ether, oleuropein hexa-TMS ether (**7**). The fragment pattern is similar to that of ligustroside penta-TMS ether (**3**) except that the molecular ion (M^+) is observed at m/z 972 in this case. The fragment ion at m/z 281 is corresponding to 3, 4-dihydroxyphenylethyl moiety (Fig. 2-[C]). Fig. 3 shows the GLC of the extractives of the inner bark and the xylem of the young shoot of yachidamo (as TMS ether derivatives). The retention times (*Rt*) of the two peaks marked as 12 (FA) and 13 (FI) are corresponding to those of ligustroside penta-TMS ether (**3**) and oleuropein hexa-TMS ether (**7**), respectively. They were confirmed by co-injection of the TMS ethers of the isolated ligustroside and oleuropein into GLC and by the analysis of the data of the gas liquid chromatography-mass spectroscopy (GC/MS).

2.3 Identification of the glycosidey Sy-X in the cambial sap of murasakihashidoi to be oleuropein

The TLC, GLC and GC/MS analyses of the cambial sap of murasakihashidoi revealed that the compound Sy-X, which was found in the cambial sap of murasakihashidoi,[5] is oleuropein (**5**).

2.4 Seasonal variations of the glycosides

Fig. 4 shows the seasonal variations of ligustroside (**1**) and oleuropein (**5**) in the inner bark and the xylem of the young shoots of yachidamo. The amount of ligustroside (**1**) in the inner bark changed in the range of 25~80 mg/g with a maximum at August and a mini-

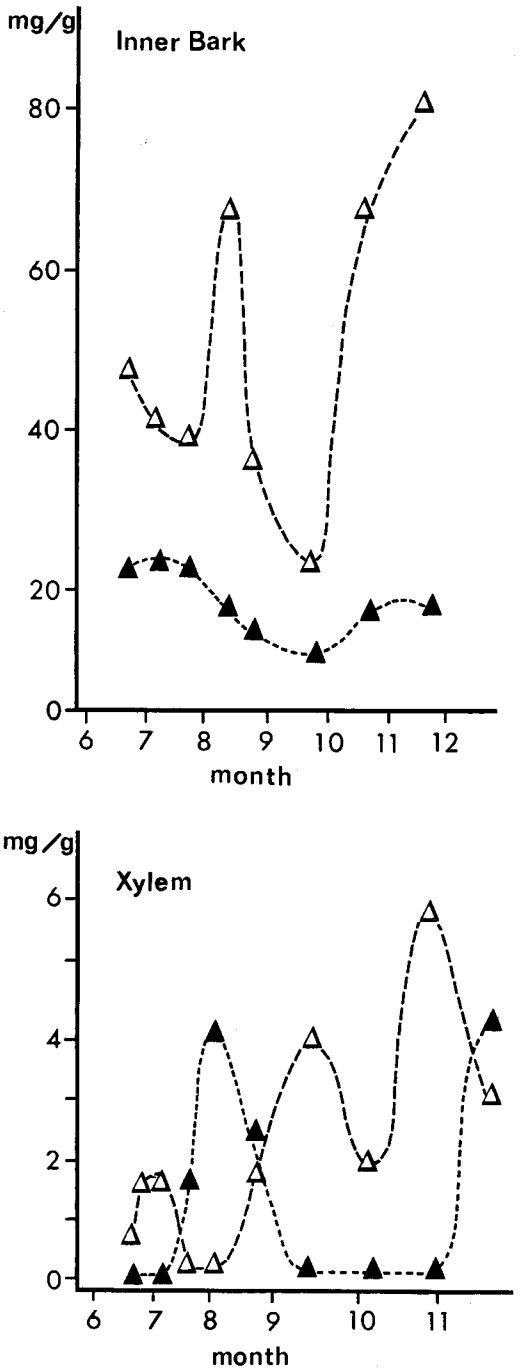


Fig. 4. Seasonal variations of ligustroside (**1**) and oleuropein (**5**) in the inner bark and the xylem of the young shoots of yachidamo.

Legends: △---△ ligustroside (**1**), ▲·····▲ oleuropein (**5**).

mum during September, and then increased toward December. Ligustroside (**1**) in the xylem showed three maxima at July, September and the end of October, and decraeased toward December. This pattern is different from those of ligustroside (**1**) in the inner bark and oleuropein (**5**) in the inner bark and in the xylem.

The amounts of oleuropein (**5**) in the inner bark of the wood were about two fifth of those of ligustroside (**1**). It showed a slight reduction between the late summer and the beginning of autumn. On the other hand, oleuropein (**5**) in the xylem was hardly detected almost all the growing season except July and early August. However, it appeared at November and increased toward winter.

3. DISCUSSION

3.1 Ligustroside and oleuropein in Oleaceae

Oleuropein (**5**) was named for the bitter principle, an unknown glycoside in olive (*Olea europea* L.) [Oleaceae] by BOURQUELOT and others in 1908.[10] Its structure was first demonstrated by PANIZZI and others.[11] INOUYE and others[12] studied the absolute configuration of oleoside (**22**), the acid glucoside counter part of the ester, oleuropein (**5**). The compound was classified as secoiridoid compound.

Ligustroside (**1**) was isolated from the leaves of ibotanoki (*Ligustrum obtusifolium* SIEB. et ZUCC.) [Oleaceae] by ASAKA and others together with 10-hydroxyligustroside (**15**).[7]

The occurrence of oleuropein (**5**) in the leaves of toneriko (*Fraxinus japonica* BLUME) [Oleaceae] was reported by INOUYE and others.[13] Ligustroside (**1**) and oleuropein (**5**) had been found in several species in Oleaceae but this is the first time to find the co-occurrence of ligustroside (**1**) and oleuropein (**5**) in the woods of *Fraxinus*[14] and *Syringa* [Oleaceae] and oleuropein (**5**) in the cambial sap of the latter.[5]

3.2 Secoiridoid glycosides in Oleaceae

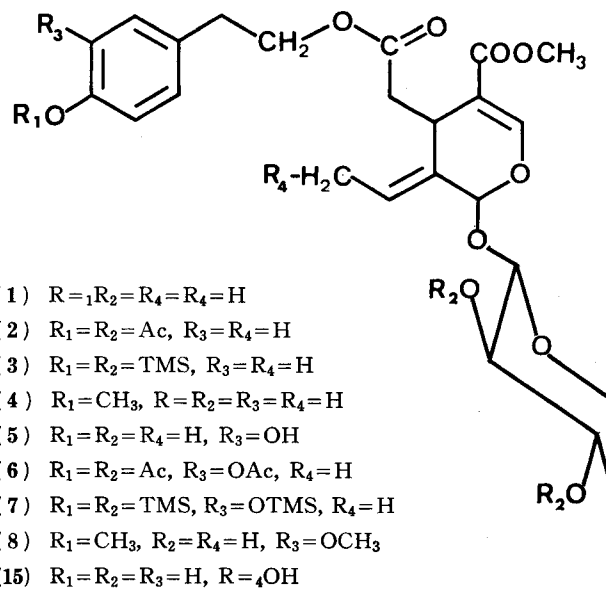
Several secoiridoid compounds were found in the species of Oleaceae in addition to the two glucosides mentioned above: nüzhenid (**14**) and oleuropein (**5**) from the ripe fruits of tonezumimochi (*Ligustrum lucidum* AIT.)[16] and nezumimochi (*L. japonica* THUM.)[15]; jusminin (**18**) from the leaves of unnansokei (*Jusminus primulinum* HASML.)[16]; 10-acetoxyligustroside (**16**) and 10-acetoxyoleuropein (**17**) from the leaves of kinmokusei (*Osmanthus fragrans* LOUR)[17]; nüzhenid (**14**), an ester (**24**) of nüzhenid and oleoside 7-methyl ester and an ester (**25**) of ligustroside and oleoside 7-methyl ester from the embryos of white ash (*Fraxinus americana*).[18]

3.3 The other iridoid glycosides in Oleaceae

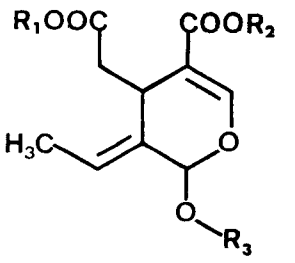
Several iridoid compounds were found in species of Oleaceae: syringopicroside (**19**) from the leaves of lilac (*Syringa vulgaris* L.)[19] and forsythid (**13**) from the leaves of shinarengyo (*Forsythia viridissima* LIDL.).[20]

3.4 Chemotaxonomic significance of the glycosides

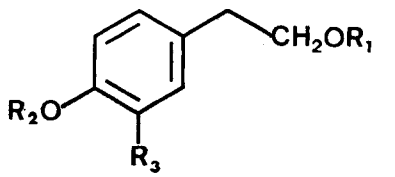
These results described above indicate that Oleaceae can be characterized by



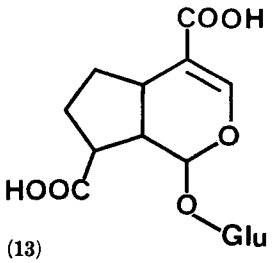
(1) $R=_1R_2=R_4=R_4=H$
(2) $R_1=R_2=Ac$, $R_3=R_4=H$
(3) $R_1=R_2=TMS$, $R_3=R_4=H$
(4) $R_1=CH_3$, $R=R_2=R_3=R_4=H$
(5) $R_1=R_2=R_4=H$, $R_3=OH$
(6) $R_1=R_2=Ac$, $R_3=OAc$, $R_4=H$
(7) $R_1=R_2=TMS$, $R_3=OTMS$, $R_4=H$
(8) $R_1=CH_3$, $R_2=R_4=H$, $R_3=OCH_3$
(15) $R_1=R_2=R_3=H$, $R=_4OH$
(16) $R^1=R_2=R_3=H$, $R_4=OAc$
(17) $R_1=R_2=H$, $R_3=OH$, $R_4=OAc$
(26) $R_1=R_2=H$, $R_3=OH$, $R_4=OH$



(20) $R_1=R_2=CH_3$, $R_3=H$
(21) $R_1=R_2=CH_3$, $R_3=Glu(OAc)_4$
(22) $R_1=R_2=H$, $R_3=Glu$
(23) $R_1=H$, $R_2=CH_3$, $R_3=Glu$



(9) $R_1=R_2=R_3=H$
(10) $R_1=Glu$, $R_2=R_3=H$
(11) $R_1=R_2=H$, $R_3=OH$
(12) $R_1=H$, $R_2=CH_3$, $R=_3OH$



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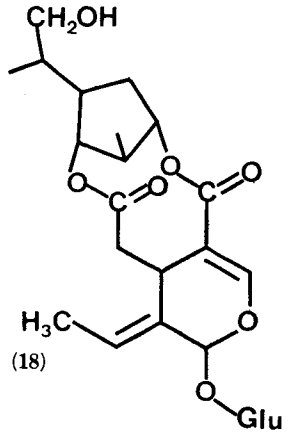
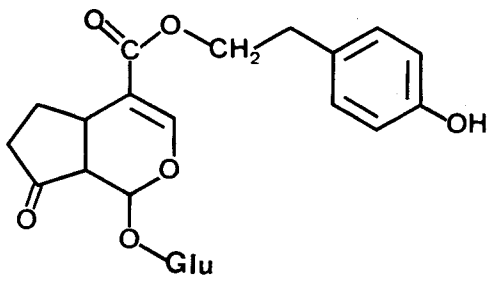


(18)



(19)

the occurrence of iridoid and secoiridoid glycosides[21] in addition to the occurrence of coumarin derivatives, which were used for chemotaxonomic tracer of the species in the genus *Fraxinus* of the family Oleaceae.[22] All of the secoiridoid glycosides found in Oleaceae contain characteristically phenylethanol derivatives in the form of esters except **13** and **18.** The ester linkages are formed at both the phenolic and alcoholic hydroxyl groups of the phenylethanol derivatives. Table 1 shows the distribution of iridoid and secoiridoid compounds in Oleaceae. Oleuropein (**5**) is most widely distributed in Oleaceae and ligustroside (**1**) and nüzhenid (**14**) follow it. Even in a same genus, the isolated secoiridois from the different organs are different (e. g. *Ligustrum*). Each genus seems to be characterized by their components. However, it is improper to discuss the chemotaxonomy of the species in Oleaceae from this table because the compounds in the table are only the isolated ones and each species might contain other compounds which were not isolated so far. The direct comparison of the extractives by TLC or GLC is necessary to discuss chemotaxonomic significance of the glycosides in detail.

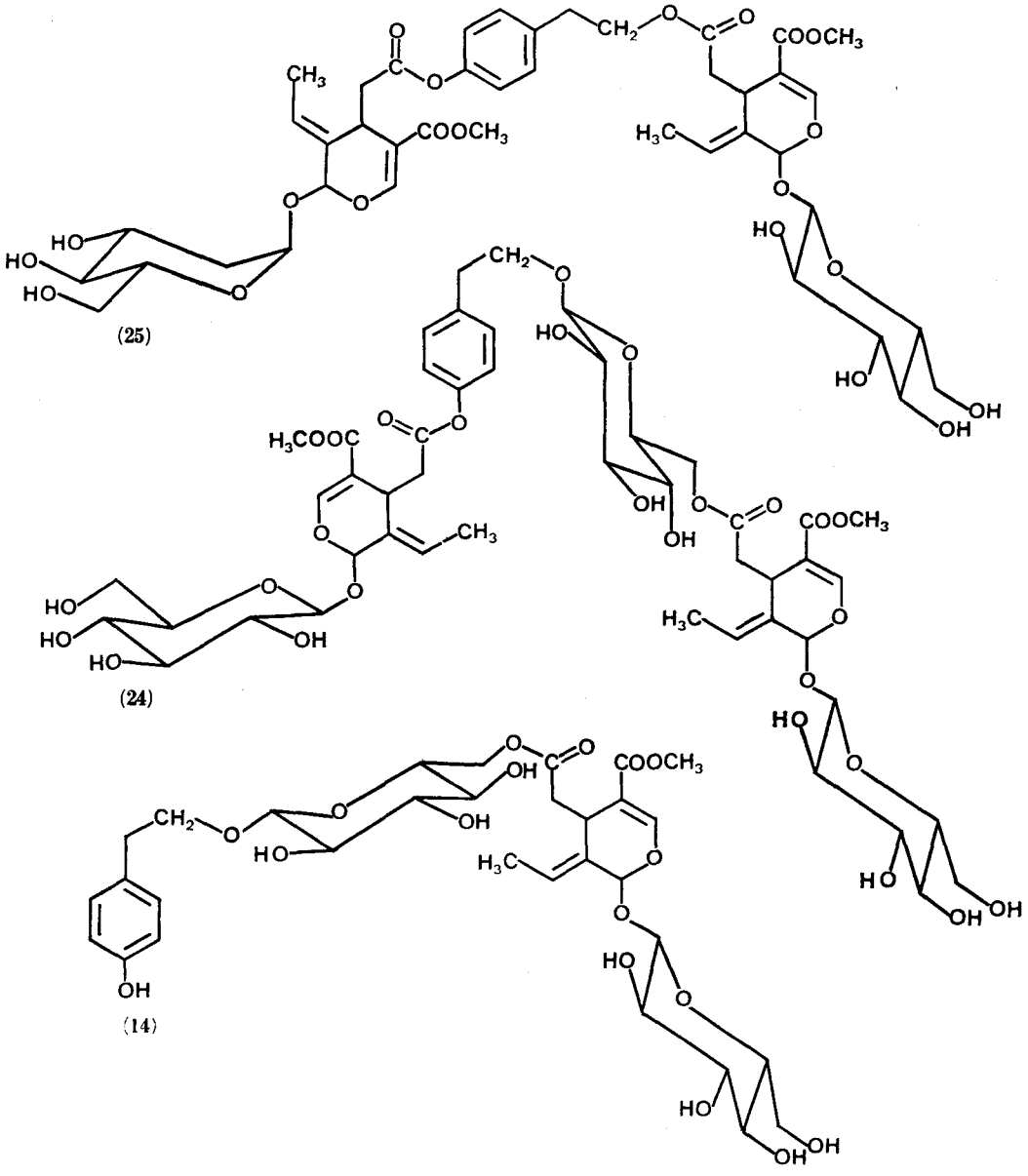
Table 1. Distribution of iridoid and secoiridoid glucosides in Oleaceae (Confirmed by their isolation)

Species in Oleaceae	Secoiridoids										Iridoids		Organs
	H (1)	HO (15)	OAc (16)	H (5)	OH (26)	OAc (17)	(14)	(18)	(24)	(25)	(13)	(19)	
O. europae L.				+									Seeds
L. obtusifolium	+	+											Leaves
L. lucidam AIT.				+			+						Fruits
L. japonica				+			+						Fruits
F. japonica Bl.				+									Leaves
F. mandshurica	+			+									IB
F. americana	+						+		+	+			Seeds
J. primilium HAS.								+					Leaves
Os. fragrans LOU.		+	+		+	+							Leaves
S. vulgaris L.	+			+									IB
S. vulgaris L.												+	Leaves
Fo. fragrans LIDL.											+		Leaves

O: *Olea*; *L*: *Ligustrum*; *F*: *Fraxinus*; *J*: *Jusminum*; *Os*: *Osmanthus*; *S*: *Syringa*; *Fo*: *Forythia*; IB: Inner bark;

3.5 Seasonal variation of the glycosides

The seasonal variations of ligustroside (**1**) and oleuropein (**5**) in yachidamo indicated that both the glucosides were not the end products but the intermediates for the other constituents unknown. The seasonal variations of the secoiridoids in the wood are rather different each other in the inner bark and the xylem. Ligustroside (**1**) changed more drastically than oleuropein (**5**) in the inner bark. On the other hand, the variation of oleuropein (**5**) in the xylem was unique because it



was observed only in summer and winter. Ligustroside (**1**) in the xylem showed three maxima during June and November, and then decreased in the middle of November. In contrast, ligustroside (**1**) in the inner bark and oleuropein (**5**) in both in the inner bark and xylem increased toward December. It will be necessary to investigate their changes during winter and toward spring.

3.6 Physiological significance of the glycosides

The relationship between the seasonal variations of these secoiridoid glycosides and the physiological phenomena of the wood is not understood well. However,

both the secoiridoid glucosides in yachidamo increased significantly toward winter after defoliation except ligustroside in the xylem. The variation patterns were similar to those of the storing materials such as starch. It was reported that the seeds of the *Fraxinus americana* are rich in lipids but do not contain starch. The seeds contain the water soluble and acid soluble glucosides in high contents instead of starch[18]. The half of the glucose obtained from the seed by acid hydrolysis was derived from the glycosides. The main glycosides were clarified to be **14**, **24**, and **25**.[18] The level of these glucosides were found to be regulated by gibberellic acid and abscisic acid in the course of the germination of the seed and the growth of the embrios.[18,25] Since the phenolic ester groups in the compounds **24** and **25** are liable to acid hydrolysis even in a mild condition such as hot methanol extraction, the extraction of these glucosides has to be treated in the temperatures lower than 10°C.[18] It is an interseting problem whether or not ligustroside (**1**) and oleuropein (**5**) exist in the form of the phenolic esters like the compound **25** and involve in the some roles for the germination of the buds in the young shoots of the wood in spring.

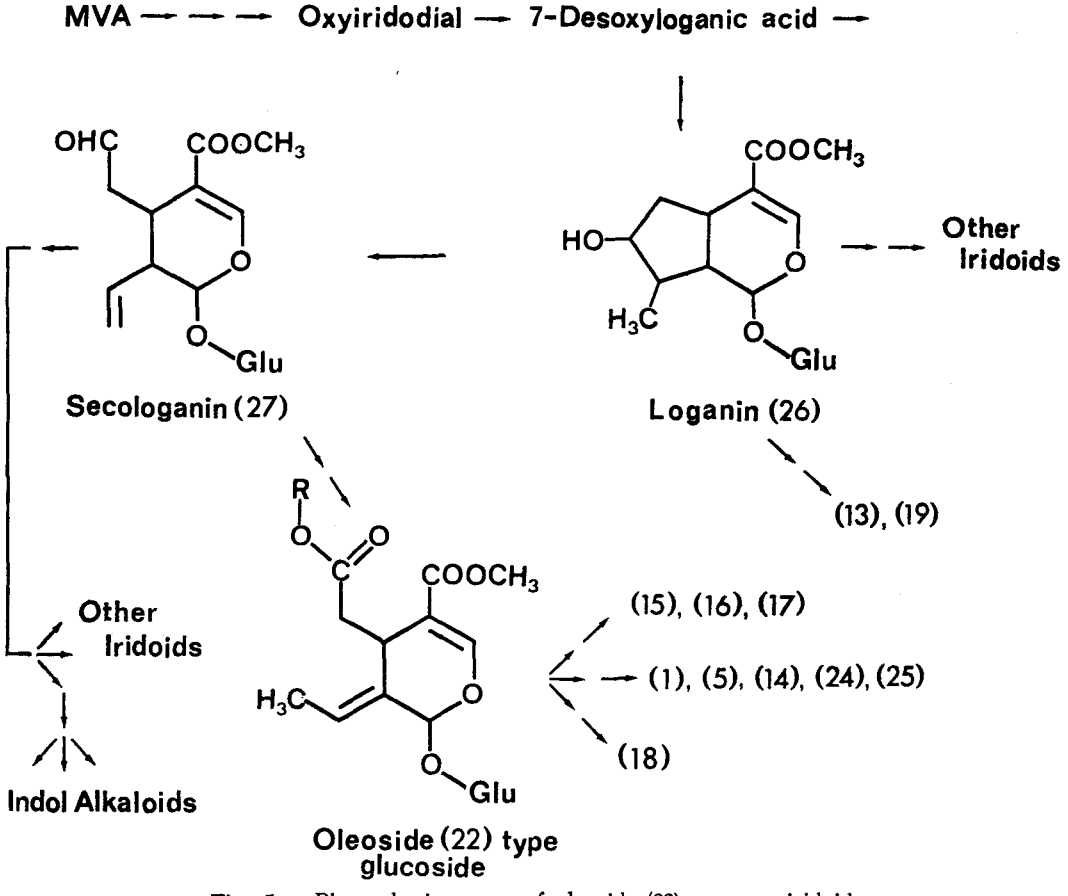


Fig. 5. Biosynthesis routes of oleoside (**22**) type secoiridoid glucosides found in Oleaceae.

3.7 Biosynthesis of oleoside type secoiridoids

Oleoside 7-methyl ester unit (**23**) of ligustroside and oleuropein was found to be biosynthesized from mevalonic acid through loganin (**26**) and secologanin (27)[26] as shown in Fig. 5. These two iridoids are very important intermediates for the other type of the iridoid compounds.[27] In addition, secologanin (**27**) was found to be the intermediate for some of indol alkaloids.[28] However, no literatures on the other types of iridoid compounds than oleoside (**22**) and indol alkaloids in the genus *Fraxinus* has been reported so far.

3.8 Glycoside Sy-X (oleuropein) in the cambial sap of murasakihashidoi

In the course of the investigation on the phenolic compounds in the cambial sap of woods,[5,23,24] it was found that the seasonal variation of the glycoside Sy-X in the cambial sap of murasakihashidoi during the growing season was so drastic as that of coniferin in the cambial sap of coniferous woods.[5] This observation lead us to study the structure of glycoside Sy-X to clarify whether or not the glycoside Sy-X was a reservor for lignin formation. However, the glycoside Sy-X is found to be a secoiridoid glucoside, oeluropein (**5**). From its structure, it seems highly unlikely that the glycoside Sy-X has relation to the lignin formation in the wood.

4. EXPERIMENTAL

4.1 Chromatographies

4.1.1 GLC: Conditions used for the quantitative periodical analyses of ligustroside (**1**) and oleuropein (**5**) were as follows: a Shimadzu GC-4BPF type GLC, 2 m glass column packed with OV-1 1.5% on Shimalite W., Temperatures: initial temperature 140°C and final temperature 290°C with program rate 3°C/min., injection temperature 250°C, detector temperature 310°C. Carrier gas: N_2 with flow rate 40 mℓ/min. Detector: FID. The conditions used for sugar analysis were the same as those described above except the column temperature with an isothermal at 160°C. Sugar samples were treated by TMS reagent before injection to GLC.

4.1.2 GC/MS: A Hitachi RMU-6MG GC/MS was used. The conditions were same as those described in the previous paper.[2]

4.1.3 TLC: Wako-gel B-10 in 250 μm in thickness. Developing solvents: SG-III: Toluene, formic acid, ethyl acetate/5 : 1 : 4 v/v; BAW: *n*-butanol, acetic acid, water/5 : 1 : 4 v/v, upper layer; AEAW: acetone, ethyl acetate, water/10 : 10 : 1 v/v. Color reagents: diazotized sulfanilic acid (DSA) in 2% sodium carbonate solution and successive spray of 50% sulfuric acid solution and heating at 105°C for 5～10 minutes.

4.1.4 PPC: Toyo-roshi No. 51. Developing solvent: BAW. Color reagent: aniline hydrogen phthalate acetic acid solution and heating at 105°C for 10 minutes.

4.2 Isolation of the glycoside FA (ligustroside) and the glycoside FI (oleuropein)

The glycosides FA and FI were isolated by silica gel column chromatography

using ethyl acetate saturated with water as a developing solvent in the same way as described in the other papers. Ligustroside (**1**, FA, 3.6 g) and oleuropein (**5**, FI, 0.6 g) were isolated from the methanol extractives (90 g) of the young shoots of yachidamo. Ligustroside (**1**, FA, 0.1 g) and oleuropein (**5**, FI, 0.3 g) were isolated from the inner bark of murasakihashidoi. The compound Sy-X found in the cambial sap of murasakihashidoi was identified to be oleuropein (**5**) by the comparison of the GLC and TLC: GLC (OV-1); *Rt* 74.6 min. (as TMS ether), TLC (AEAW): *Rf* 0.63.

4.3 Physico-chemical properties of the glucosides and their derivatives

The UV, IR and NMR spectroscopies were the same as those described in the previous papers.[2,23,24]

4.3.1 Ligustroside (**1**), FA: The glucoside FA, ligustroside (**1**) was obtained as pale yellow powder but pure on TLC (AEAW) with *Rf* 0.72. Color reaction: orange with DSA and changed into green by the treatments with 50% sulfuric acid spray and heating. Ultra violet (UV) absorption λ_{max}^{EtOH}. nm: 222, 280, 284(s), shifted to 244, 289 in alkaline medium. IR ν_{max}^{KBr}. cm^{-1}: 3350 (OH), 285~2950 (CH), 1730 (-COOR), 1705 (>C=CH-COOR), 1460, 1435, 1380, 1350, 1200, 1160, 1110, 1070, 1010, 940, 920, 900, 830, 820.

4.3.2 Ligustroside pentaacetate (**2**): Ligustroside (**1**, 155 mg) was treated with acetic anhydride and pyridine at 55°C for 12 hours. The acetate was obtained as paste state (180 mg). *Anal.* Calcd. for $C_{35}H_{42}O_{17}$: C 56.65; H 5.68. Found: C 57.28; H 5.97. ^{1}H-NMR (10% in $CDCl_3$) δ (ppm): 7.49 (1 H, *s.*, -O-CH=C<), 7.16 (4 H, *d. d.*, AA'BB' system, Ar-H of 4-AcOPh-CH_2-), 5.98 (1 H, *q.*, CH_3-C*H*=C<), 5.74 (1 H, *m.*, =C-C*H*<O-O-), 3.75 (3 H, *s.* -$COOCH_3$), 4.24 (2 H, *t.*, J=7.5 Hz, Ar-CH_2-C*H*$_2$O-), 2.29 (2 H, *t.*, J=7.5 Hz, Ar-C*H*$_2$-CH_2O-), 2.32 (3 H, *s.*, Ar-$OCOCH_3$), 2.05 (12 H, *s.*, four alc-$OCOCH_3$), 1.69 (3 H, J=8, 2 Hz, C*H*$_3$-CH=C<), 4.9~5.3 and 3.8~4.2 (7 H, one-C*H*$_2$OCOCH_3, four-C*H*OCOCH_3- in Glu). MS (20 eV) *m/z*: 734 (M^+), 692 (M^+-42), 555 (oleoside residue), 526, 513, 403 (aglycon ester residue), 387, 386, 355, 345, 316, 331 (Glu), 289, 271, 229, 225 (acid residue), 223, 163 (4-AcOPh$CH_2CH_2^+$), 211, 169, 145, 190, 103, 73.[8] IR ν_{max}^{KBr}. cm^{-1}: 2960 (CH), 1760 (COOR), 1729 (COOR), 1640 (CH=CH), 1510 (Ph), 1440, 1360, 1300, 1220, 1165, 1100, 1070, 1040, 910, 855, 815, 760.

4.3.3 Ligustroside penta-TMS ether (**3**): FA was treated with TMS reagent and subjected to GLC (OV-1). A single peak was observed at *Rt* 71.3 min. MS (20 eV) *m/z*: 884 (M^+, missing), 869 (M^+-15), 506, 490, 451 (Glu), 417 (aglycon ester resid.), 387, 361 (451-90), 331, 319, 271, 243, 217, 193 (4-TMSOPhCH_2-CH_2^+), 169, 129, 103, 73 (Fig. 3).

4.3.4 Ligustroside monomethyl ether tetraacetate (**4**): FA was treated with diazomethane (CH_2N_2) overnight followed by acetylation with acetic anhydride and pyridine at 55°C for 8 hr. Ligustroside monomethyl ether tetraacetate (**4**) was obtained as yellow paste. ^{1}H-NMR (10% in $CDCl_3$) δ (ppm): 7.43 (1 H, *s.*, -OOC-C=CH-O-), 6.95 (1 H, *m.*, CH_3-C*H*=C<), 5.79 (1 H, *m.*, =C-C*H*<O-O-), 4.16 (2 H,

t., J=8 Hz, Ar-CH_2-CH_2O-), 3.79 (3 H, *s.*, $ArOCH_3$), 3.72 (3 H, *s.*, -$COOCH_3$), 2.85 (2 H, *t.*, J=8 Hz, Ar-CH_2-CH_2O-), 2.08 (12 H, *d.*, four alc-$OCOCH_3$), 1.68 (3 H, *d. d.*, J=8, 2 Hz, CH_3-CH=).

4. 3. 5 Oleoside dimethyl ester tetraacetate (**21**): FA ligustroside (**1**) was hydrolyzed with 0.1 *N* NaOH ethanol solution at room temperatures for 60 min. Ether was added to the hydrolyzates and the precipitates produced were dissolved into water. The water solution was neutralized with Amberlite IR-120 (H^+ form) to remove Na^+. The dried hydrolyzates were separated by silica gel column chromatography using ethyl acetate saturated with water as a developing solvent. 2-(4′-Hydroxyphenyl)ethanol, tyrosol (**9**) was isolated from the earlier eluates and a more polar compound (**22**) with *Rf* 0.31 on TLC (BAW) was isolated from the middle of the eluates. The latter compound was treated with CH_2N_2 and purified by silica gel column chromatography using the same solvent system mentioned above. The purified methyl ester was acetylated with acetic anhydride and pyridine in the same way as described in **4. 2. 2.** The acetate (**21**) was obtained as paste state but pure on TLC (SG-III). The same compound **21** was derived through the acid glucoside (**22**) obtained from FI, oleuropein (**5**) by the same procedures. ^{1}H-NMR (10% in $CDCl_3$, 100 MHz) δ (ppm): 1.75 (3 H, *d. d.*, J=8, 2 Hz, CH_3-CH=C<), 2.02 (9 H, *s.*, three alc-$OCOCH_3$), 2.07 (3 H, *s.*, one alc-$OCOCH_3$), 2.40 (1 H, *d. d.*, J=14, 4 Hz, -OOC-C$H_A$$H_B$-$CH_X$-), 2.76 (1 H, *d. d.*, J=14, 10 Hz, -OOC-$CH_A$$H_B$-$CH_X$-), 3.69 (3 H, *s.*, -$COOCH_3$), 3.98 (1 H, *d. d.*, J=10, 4 Hz, -OOC-$CH_A$$H_B$-C$H_X$-), 4.0～4.5 (2 H, *m.*, -CH_2-$OCOCH_3$), 4.9～5.3 (4 H, *m.*, four -CH-$OCOCH_3$-), 5.74 (1 H, *s.*, =C-CH-O-), 6.03 (1 H, *q.*, J=8 Hz, CH_3-CH=C<), 7.84 (1 H, *s.*, >C=CH-O-). The decoupling at δ 6.03 made the doublet at δ 1.75 a singlet and the decoupling at δ 3.98 made the two quartets at δ 2.40 and 2.76 to corresponding doublets. The decoupling at δ 2.56 made the doublets at δ 3.98 a singlet (Fig. 1). IR ν_{max}^{KBr} cm^{-1}: 2850～2960 (CH), 1760 (COOR), 1700 (COOR), 1635 (C=C), 1460, 1375, 1350, 1305, 1260, 1230, 1210, 1165, 1120, 1100, 1090, 1070, 1060, 1040, 985, 925, 910.

4. 3. 6 2-(4-Hydroxyphenyl)ethanol, tyrosol, (**9**): Tyrosol (**9**) was isolated from the mild alkaline hydrolyzates of ligustroside (**1**) by silica gel column chromatography as described in **4. 2. 5.** Colorless crystals with mp 91～92°C was obtained. The same compound was obtained from the acid hydrolyzate of ligustroside (**1**) and from the extractives of the inner bark of the same wood as free state. UV λ_{max}^{EtOH}. nm: 280, 284 (s). IR ν_{max}^{KBr}. cm^{-1}: 3380～3140 (OH), 2950～2850 (CH), 1608 (Ph), 1692 (Ph). 1510 (Ph), 1470, 1445, 1360, 1340, 1238, 1228, 1180, 1168, 1124, 1100, 1050, 1010, 970, 950, 930, 865, 815, 785, 730, 710. ^{1}H-NMR (10% in d_6-acetone) δ (ppm): 6.90 (4 H, *d. d.*, J=8 Hz, AA′BB′ system, Ar-H of 4-$OHPhCH_2$-). 3.55 (2 H, *t.*, J=7 Hz, Ar-CH_2-CH_2O-), 2.74 (2 H, *t.*, J=8 Hz, Ar-CH_2-CH_2O-). *Anal.* Calcd. for $C_8H_{10}O_2$: C 69.54; H 7.30. Found: C 69.20; H 7.43. TLC (SG-III): *Rf* 0.61.

4. 3. 7 Oleuropein (**5**), FI: The glycoside FI, oleuropein (**5**) was obtained as white powder and pure on TLC (AEAW) with *Rf* 0.63. Color reaction: pinkish orange with DSA and changed to reddish orange by successive spraying of 50%

sulfuric acid solution and heating. The chromatographic behaviors on TLC and GLC were identical with those of the authentic specimen.

4. 3. 8 Oleuropein hexaacetate (**6**): Oleuropein hexaacetate (**6**): Oleuropein (**5**, 154 mg) gave an acetate (210 mg) by acetylation with acetic anhydride and pyridine in the same way as described in **4. 2. 2.** ^{1}H-NMR (10% in $CDCl_3$) δ (ppm): 7.46 (1 H, *s.*, -OOC-C(=)CH-O-), 7.05~7.03 (3 H, *m.*, Ar-H), 5.98 (1 H, *q.*, J=8 Hz, CH_3-C*H*=C(=), 4.22 (3 H, *t.*, J=7 Hz, Ar-CH_2-C*H*$_2$O-), 2.98 (2 H, *t.*, J=7 Hz, Ar-C*H*$_2$-CHO_2-), 3.72 (3 H, *s.*, -$COOCH_3$), 2.28 (6 H, *s.*, two Ar-$OCOCH_3$), 2.05 (12 H, *s.*, four alc-$OCOCH_3$), 1.68 (3 H, *d. d.*, J=7.0, 1.5 Hz, C*H*$_3$-CH=C(=), 5.3~5.0 and 4.2~4.0 (7 H, *m.*, one -C*H*$_2$O$COCH_3$, four -C*H*O$COCH_3$- of Glu.). MS (20 eV) m/z: 792 (M^+) 750 (M^+-42), 732 (M^+-60 (AcOH)), 708 (M^+-42×2), 690 (M^+-60-42), 672 (M^+-60×2), 555, 526, 461, 460, 445, 444, 413, 403, 371, 360, 331 (Glu), 289, 271, 229, 225, 223, 221, 207.[8]

4. 3. 9 Oleuropein hexa-TMS ether (**7**): Oleuropein (**5**) was treated with TMS reagent and a TMS ether (**7**) was obtained and subjected to GLC (OV-1). A single peak was observed at *Rt* 74.6 min, which was identical with that of the authentic specimen. MS (20 eV) m/z: 972 (M^+), 594, 505 (aglycon ester resid.), 451 (Glu.), 361 (451−90), 281 ($^+CH_2$-CH_2-Ar(OTMS)$_2$), 193, 191 (Fig. 3).

4. 3. 10 Oleuropein dimethyl ether (**8**): Oleuropein (**5**) was treated with CH_2N_2 and a methyl ether (**8**) was obtained as pure state on TLC (AEAW) with *Rf* 0.67. No color with DSA, detected by 50% sulfuric acid solution and heating. The dimethyl ether was subjected to alkaline hydrolysis in the same way as described in **4. 3. 5.**

4. 3. 11 2-(3′, 4′-Dihydroxyphenyl)ethanol (**11**): The glycoside FI, oleuropein (**5**) was treated with 0.1 *N* NaOH solution at 95°C for 15 minutes. The reaction mixture was acidified with HCl and extracted with ethyl ester. The ether soluble fraction was purified by silica gel column chromatography using *n*-hexane and ethyl acetate (3 : 1~1 : 1 v/v, gradient) and 2-(3′, 4′-dihydroxyphenyl)ethanol (**11**) was obtained as paste state but pure on TLC (SG-III) with *Rf* 0.52. Color reaction: orange with DSA and changed to reddish orange by successive spraying 50% sulfuric acid solution. The chromatographic behavior on TLC was same as that of 2-(3′, 4′-dihydroxyphenyl)ethanol obtained from the inner bark of yachidamo before.

4. 3. 12 2-(3′,4′-Dimethoxyphenyl)ethanol (12): 2-(3′,4′-Dihydroxyphenyl)ethanol (**11**) was methylated with CH_2N_2 and a methyl ether (**12**) was obtained. The same compound (**12**) was also obtained by hydrolysis of oleuropein dimethyl ether (**8**) in the same way as described in **4. 3. 5** and the ether soluble hydrolyzate was purified by silica gel column chromatography using *n*-hexane and ethyl acetate. TLC (SG-III): *Rf* 0.47. ^{1}H-NMR (10% in $CDCl_3$) δ (ppm): 6.78 (3 H, *m.*, Ar-H), 3.82 (3 H, *s.*, Ar-OCH_3), 3.84 (3 H, *s.*, Ar-OCH_3), 3.80 (2 H, *t.*, J=8 Hz, Ar-CH_2-C*H*$_2$O-), 2.80 (2 H, *t.*, J=8 Hz, Ar-C*H*$_2$-CH_2O-).

4. 3. 13 Glucose, the glycosyl residue of both the secoiridoid glycoside, ligustroside (**1**) and oleuropein (**5**): Hydrolysis of ligustroside (**1**) and oleuropein (**5**) with 3% sulfuric acid solution yielded glucose as a glycosyl residue of the two com-

pounds. PPC (BAW): *Rf* 0.18. GLC (OV-1): *Rt* 17.93 (α), 30.03 (β) min. as TMS ether. The data were identical with those of the authentic glucose.

4.4 Periodical analysis of the glycosides

The young shoots of yachidamo (grown in Obihiro) were collected periodically and extracted with ethyl alcohol and extracts were evaporated to dryness and weighed. A part of the extract was treated with TMS reagent using raffinose as an internal standard and subjected to GLC.

5. CONCLUSION

The glycosides FA and FI found in the extractives from the inner bark of yachidamo (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.) were identified to be secoiridoid glucosides, ligustroside (**1**) and oleuropein (**5**), respectively. The cooccurrence of the glucosides **1** and **5** was also found in the inner bark of murasakihashidoi (*Syringa vulgaris* L.).

The compound Sy-X in the cambial sap of murasakihashidoi is found to be oleuropein (**5**) and to have no relation to the lignin formation in the wood.

The seasonal variations of these glucosides in the young shoots of yachidamo indicated that these compounds increased toward winter after defoliation.

The family Oleaceae can be characterized by secoiridoid glycosides. Most of the secoiridoids glucosides contain a phenylethanol moiety as a counterpart of the ester. Further investigation is necessary to discuss the chemotaxonomic significance of the secoiridoid glycosides in Oleaceae in detail.

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要　　約

モクセイ科のヤチダモおよびムラサキハシドイの内樹皮から単離した成分の同定および季節変化について研究した。

ヤチダモ (*Fraxinus mandshurica* RUPR. var. *japonica* MAXIM.) の内樹皮から単離された配糖体 FA および FI は，それぞれセコイリドイド配糖体である ligustroside (**1**) と oleuropein (**5**) と同定された。

これら ligustroside (**1**) と oleuropein (**5**) が，ムラサキハシドイ (*Syringa vulgaris* L.) の内樹皮中にも共存していることが両者を単離することで確認された。

ヤチダモ若枝中の内樹皮および材部での含有量の変化を GLC 法により調べた。ligustroside (**1**) は，内樹皮中で 25～80 mg/g の間で変化した。8 月に極大値を示したのち，9 月から 10 月にかけて極小値を示し，次いで 12 月に向けて大きく増加した。材中の ligustroside (**1**) は，内樹皮中のそれの 1/10 以下で増減をくりかえし，10 月下旬で最大となった。一方，oleuropein (**5**) は，ligustroside (**1**) の 2/5～1/3 程度の含有量で，内樹皮中では年間を通じて大きな変化は

なかった。9月，10月にわずかに減少した。材中の oleuropein (5) は，7月，8月以外はほとんど検出されず，11月に出現し，12月に向けて増加した。

ムラサキハシドイの形成部位の樹液中にあって，生長期に増減の激しかった配糖体 Sy-X は，針葉樹の樹液中の coniferin の季節変化に類似していたことから興味がもたれていた。しかしながら，本研究によって，oleuropein (5) と同定されたことから，リグニン形成とは直接関係する化合物ではないことが明らかとなった。

ligustroside (1) や oleuropein (5) を含むセコイリドイド配糖体の生合成，モクセイ科での分布，chemotaxonomy のための指標成分としての評価，樹体内や，種子内でのこれらの成分および関連化合物の生理的役割について論じた。