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The phenolic constituents of normal and reaction woods of Karamatsu, *Larix leptolepis* GORD*.

By

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カラマツ正常材およびあて材のフェノール性成分*

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1. Introduction

Chemical studies on compression wood, which is developed on the lower side of a leaning stem or a branch, have mainly been carried out on hemicellulose and lignin. The former contains more galactose residue^{1,2)} than normal wood, and MEIR³⁾ has first isolated galactan consisting of (1→4)-linked β -D-galactopyranose units, which contained 13% of uronic acid residues. The nature and attachment of the uronic acid residues could not be fully elucidated, but TIMELL⁴⁾ showed that the residues had linked C-6 of a main chain of galactose. TIMELL has, furthermore, isolated xylan⁵⁾ and galactoglucomannan⁶⁾ similar in structure to hemicellulose in normal wood and laricin⁷⁾ consisting of (1→3)-linked β -D-glucan which is characteristic in compression wood.

On the other hand, compression wood has long been known to have a considerably different composition from that of normal wood, and thus the main characteristics of the former are a much higher content of lignin and a lower proportion of methoxyl groups. Systematic studies on the structure of lignin in compression wood were carried out by SAKAKIBARA⁸⁾, who pointed out that the degree of condensed units of it was larger than that of lignin in normal wood

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calculated from the NMR spectrum, suggesting that the former would have more condensed type aromatic rings than the latter. The various degradation products^{9,10} obtained by permanganate and nitrobenzene oxidations, ethanolysis and hydrogenolysis indicated that the lignin in compression wood had more condensed units of *p*-hydroxyphenyl and guaiacyl nuclei than that of normal wood. Similar result of permanganate oxidation was also obtained by MIKSCHE¹¹.

On the work of extractives⁸ of normal and compression woods of Todomatsu, *Abies sachalinensis* MAST., the latter had more total amounts of ether, ethanol-benzene, ethanol and acetone-water extracts. Particularly, difference of the ether extract contents between them is remarkable, but their constituents are the same, judging from the data of paper chromatography.

This paper is concerned with chemical transformation of extractives of Kar-amatsu wood on the way to the formation of compression wood and heartwood.

2. Experimental

All the melting points are uncorrected. The IR spectra were measured using a Hitachi 215 type spectrophotometer. The NMR spectrum was recorded on a Hitachi R-22 spectrometer, TMS being used as an internal standard. The Mass spectra were measured using a Hitachi K-53 GC spectrometer. The homogeneity and identification with authentic sample of each compound was always checked by thin layer chromatography and the spots were detected with iodine and diazotized sulfanilic acid indicators.

2.1 Extraction.

Normal wood was separated into heart- and sapwoods. Compression and side woods, both of which are sapwood, were collected from horizontally growing stem as shown in Figure 1. The above trees were collected at Tomakomai college experiment forest of Hokkaido University. Four kinds of the dried wood meal were extracted three times with methanol at room temperature, and the methanol extracts were concentrated below 45°C under reduced pressure. The residues were then percolated with *n*-hexane, benzene and ethyl acetate, successively. These solutions dried over sodium sulfate were evaporated under reduced pressure to afford syrups.

2.2 Isolation of constituents.

n-Hexane soluble fraction was chromatographed on a silica gel column and eluted successively with *n*-hexane-benzene (1:1), *n*-hexane-chloroform (3:7) and *n*-hexane-ethyl acetate (9:1) to yield 270-290 mg of lignoceryl ferulate (I) from sap- and heartwoods,

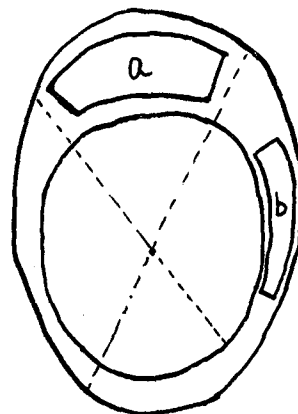


Fig. 1. Location of zones in stem containing compression wood (sapwood)
a; compression wood.
b; side wood.

and 30–35 mg of I from compression and side woods.

Benzene and ethyl acetate soluble fractions were also separated by column chromatography on silica gel. Elution with mixed solvent of *n*-hexane-benzene, benzene-ethyl acetate, chloroform-ethyl acetate and *n*-hexane-ethyl acetate, changing solvent ratio, afforded pinocembrin (II), naringenin (III), aromadendrin (IV), taxifolin (V) and kaempferol (VI). The amount of compounds (II and VI) was 240–250 mg from sap- and heartwoods, and that of III, IV and V was 21, 23 and 27 g from heartwood, respectively, and about 10 g of these three compounds from sapwood. The yields of these compounds from other soluble fraction were similar to those of sapwood.

Benzene soluble fraction from compression wood was chromatographed on silica gel using benzene as eluant to give 250 mg of *p*-hydroxybenzaldehyde (VII). Same treatment of other benzene soluble fraction furnished 200–250 mg and 50 mg of crude compound (VII) from heart- and sapwoods and side wood, respectively.

2.3 Lignoceryl ferulate (I).

Colorless needles from a mixed solvent of *n*-hexane and benzene; mp 67–68.5°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3500, 1725, 1640, 1600; MS m/e : 530 (M^+), 489, 193, 177; NMR (CDCl_3) τ : 9.08 (3H, t, $J=5$ Hz), 8.73 (4H, s), 6.08 (3H, s), 5.81 (2H, t, $J=6$ Hz), 3.71 (1H, d, $J=16$ Hz), 3.00 (3H, m), 2.37 (1H, d, $J=16$ Hz). Anal. Calcd. for $\text{C}_{34}\text{H}_{58}\text{O}_4$: C, 76.93; H, 11.01, Found: C, 77.20; H, 10.97.

2.4 Pinocembrin (II).

Colorless needles from a mixed solvent of *n*-hexane and benzene; mp 195–197°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3100, 1630, 1600; MS m/e : 256 (M^+), 197, 152, 124; NMR (CD_3OD) τ : 7.28 (1H, *q*, $J=16+4$ Hz), 6.92 (1H, *q*, $J=16+12$ Hz), 5.20 (2H, s), 4.55 (1H, *q*, $J=12+4$ Hz), 4.05 (2H, AB *q*, $J=2$ Hz), 2.55 (5H, m). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{O}_4$: C, 70.30; H, 4.72, Found: C, 70.11; H, 4.52.

2.5 Naringenin (III).

Colorless needles from a mixed solvent of benzene and tetrahydrofuran; mp 248–251°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3200, 1630, 1600; MS m/e : 272 (M^+), 152, 121; NMR (CD_3OD) τ : 7.38 (1H, *q*, $J=16+4$ Hz), 6.95 (1H, *q*, $J=16+12$ Hz), 5.20 (3H, s), 4.72 (1H, *q*, $J=12+4$ Hz), 4.12 (2H, s), 3.22 (2H, d, $J=9$ Hz), 2.72 (2H, d, $J=9$ Hz). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{O}_5$: C, 66.17; H, 4.44, Found: C, 66.01; H, 4.14.

2.6 Aromadendrin (IV).

Colorless needles from a mixed solvent of benzene and tetrahydrofuran: mp 224–226°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3450, 1650, 1600; MS m/e : 288 (M^+); NMR (CD_3OD) τ : 5.50 (1H, d, $J=12$ Hz), 5.20 (4H, s), 5.05 (1H, d, $J=12$ Hz), 4.06 (2H, AB *q*, $J=2$ Hz), 3.15 (2H, d, $J=9$ Hz), 2.65 (2H, d, $J=9$ Hz). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{O}_6$: C, 66.26; H, 4.01, Found: C, 66.25; H, 4.01.

2.7 Taxifolin (V).

Colorless needles from a mixed solvent of benzene and tetrahydrofuran: mp

242–243°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3400, 1640, 1590; MS m/e : 304 (M^+), 166, 124; NMR (CD_3OD) τ : 5.50 (1H, d, $J=11$ Hz), 5.20 (5H, s), 5.10 (1H, d, $J=11$ Hz), 4.08 (2H, AB q , $J=2$ Hz), 3.10 (3H, m). Anal. Calcd. for $\text{C}_{15}\text{H}_{12}\text{O}_7$: C, 59.21; H, 3.98, Found: C, 59.01; H, 4.03.

2.8 Kaempferol (VI).

Yellow needles from a mixed solvent of benzene and tetrahydrofuran: mp 276–277.5°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3250, 1660, 1610; MS m/e : 286 (M^+), 153, 121; NMR (CD_3OD) τ : 5.20 (4H, s), 3.80 (1H, d, $J=2$ Hz), 3.60 (1H, d, $J=2$ Hz), 3.08 (2H, d, $J=9$ Hz), 1.90 (2H, d, $J=9$ Hz). Anal. Calcd. for $\text{C}_{15}\text{H}_{10}\text{O}$: C, 62.94; H, 3.52, Found: C, 62.82; H, 3.38.

2.9 *p*-Hydroxybenzaldehyde (VII).

Colorless needles from a mixed solvent of benzene and ethyl acetate: mp 110–112°C; IR $\nu_{\max}^{\text{nujol}}$ cm^{-1} : 3100, 1660, 1590; MS m/e : 122 (M^+), 121, 93; NMR (CDCl_3) τ : 5.20 (1H, s), 3.08 (2H, d, $J=9$ Hz), 2.24 (2H, d, $J=9$ Hz), 0.30 (1H, s). Anal. Calcd. for $\text{C}_7\text{H}_6\text{O}_2$: C, 68.84; H, 4.95, Found: C, 68.57; H, 4.77.

3. Results and discussion

Yields of fractions obtained by extraction of methanol soluble part of four zones with *n*-hexane, benzene and ethyl acetate are listed in Table 1. Heartwood contained more total amount of extractive than sapwood. Furthermore, yield of each fraction from side wood had similar value to that of sapwood, and with the consideration of the fact that difference of constituents was quantitatively as well as qualitatively not observed between them as described in experimental, side wood appears to possess essentially normal wood characteristics on extractives. On the other hand, besides its high total extractives content, compression wood is characterized by its higher yields of benzene and ethyl acetate soluble fractions than those of sapwood and side wood. SAKAKIBARA and MOROHOSHI⁸⁾ have discussed about the extractives of compression wood of Todomatsu (*Abies sachalinensis* MAST.), and found that yield of ether soluble fraction from compression wood was about twofold as much as that from normal wood (opposite wood was used for comparison). In above studies, wood meal was extracted with ether, ethanol-benzene and ethanol, successively. The yields¹⁰⁾ of fractions obtained by treatment in the same way as in Todomatsu were 0.92, 3.82 and 1.10%, respec-

Table 1. Yields of fractions obtained by extraction

Sample	Milled wood (Kg)	<i>n</i> -Hexane soluble fraction (%)	Benzene soluble fraction (%)	Ethyl acetate soluble fraction (%)
Normal heartwood	29.6	0.48	0.25	2.16
Normal sapwood	25.0	0.62	0.04	0.37
Compression wood	4.0	0.36	0.13	0.78
Side wood	3.0	0.42	0.07	0.30

tively. The corresponding figures from the side wood were 1.19, 2.54 and 0.29%, respectively. SAKAKIBARA and MOROHOSHI⁸⁾ also discussed the results from tension wood of Yachidamo (*Fraxinus mandshurica var. japonica*) and lead to the conclusion that the stimulative factors of formation of reaction wood were associated with the change of metabolism which would increase the extractives on the lower side of a leaning stem or a branch. Karamatsu wood seems to have similar tendency to above phenomena except for difference of chemical characteristics on extractives.

n-hexane soluble fraction was separated by column chromatography on silica gel to isolate a compound (I), which was crystallized from a mixed solvent of *n*-hexane and benzene as colorless needles, mp 67–68.5°C and analyzed for C₃₄H₅₈O₄. In the IR spectrum, the compound (I) possessed hydroxyl, ester, olefinic and aromatic functions since it exhibited absorptions at 3500, 1725, 1640 and 1600 cm⁻¹. The NMR spectrum showed three aromatic protons at τ 3.00 (3H, m) and two olefinic protons at τ 3.71 (1H, d, $J=16$ Hz) and 2.37 (1H, d, $J=16$ Hz), pointing out that the presence of ferulate in molecule, and furthermore, methyl group at τ 9.08 (3H, t, $J=5$ Hz) and methylene signals at τ 8.74 (4H, s) and 5.81 (2H, t, $J=6$ Hz), arising from a lignoceryl function. The above data which were completely identical with those of an authentic specimen of lignoceryl ferulate, have enabled us to propose the structure of I for the compound.

Lignoceryl ferulate has already been isolated from Ohyonire (*Ulmus laciniata* MAYR)¹²⁾ and Akinire (*Ulmus parvifolia* JACQ.)¹³⁾, in both of which the compound (I) was only isolated from sapwood, but not detected in heartwood by thin layer chromatography, suggesting the chemical change on extractive on the way to the formation of heartwood. However, such a phenomenon was not observed in Karamatsu, which contained I in all zones. Lignoceryl ferulate has, by this time, not been isolated from *Larix leptolepis* Gord., but eicosanyl and other ferulates have been isolated from *Larix laricina*¹⁴⁾ grown in Canada and *Larix lyallii*¹⁵⁾, respectively.

Compounds (II and III) were estimated to be flavonoid from the IR and NMR spectra. Moreover, the NMR spectrum showed three quartets at τ 7.28, 6.92 and 4.55 in II and at τ 7.38, 6.95 and 4.72 in III, indicating that both compounds have a partial structure of $-\text{CH}-\text{CH}_2-\text{C}-$ as C₃ part of C₆-C₃-C₆ skeleton.



The signals of AB system, which appeared at τ 4.05 with coupling constant $J=2$ Hz (2H) in the NMR spectrum of II and at τ 4.05 (2H) with the same coupling constant in that of III, are attributed to the aromatic protons of A ring and indicate *meta* disposition of two protons. Accordingly, there is a structural difference between them in B ring, namely, a multiplet at τ 2.55 (5H) appears in the NMR spectrum of II and two doublets with coupling constant $J=9.0$ Hz at τ 3.22 (2H) and 2.72 (2H) in that of III. The IR spectrum of III was completely identical with that of naringenin¹⁶⁾ indicating that II is pinocembrin. The Mass spectra of both compounds supported these structures. Pinocembrin and naringenin

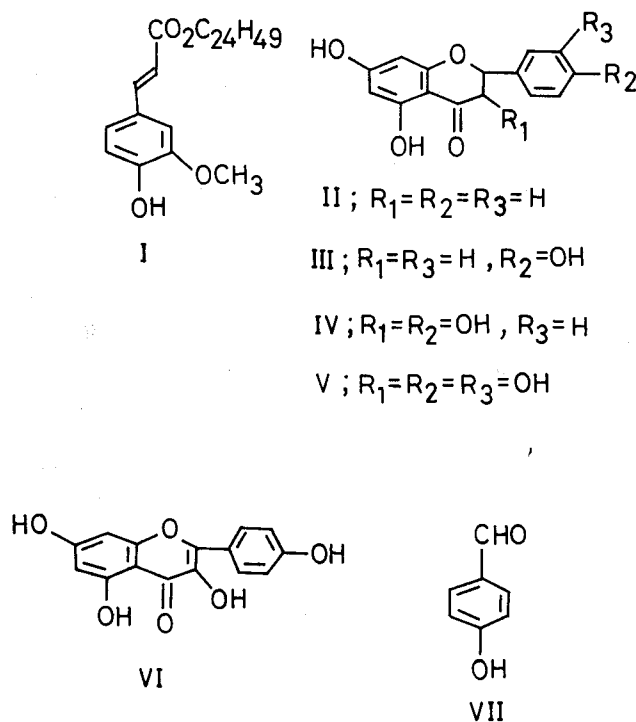


Fig. 2. The compounds isolated from Karamatsu.

was isolated from the genus *Larix* for the first time.

Aromadendrin (IV), taxifolin (V) and kaempferol (VI) have already been isolated and/or checked up on the presence in the same wood by SASAYA *et al*¹⁷⁾. The amounts of naringenin (III), aromadendrin (IV) and taxifolin (V) are dominant in Karamatsu wood among above five compounds, and a phenomenon is remarkable in heartwood compared with other zones.

The compound (VII) isolated only from benzene soluble fraction of compression wood was estimated to be a phenolic substance which may possess an unsaturated carbonyl function judged from the IR spectrum. The NMR spectrum of VII exhibited that two substituents, hydroxyl and formyl (τ 0.30 (1H, s)) groups, on the aromatic ring have *para* disposition, judging from two doublets at τ 3.08 (2H) and 2.24 (2H) with couplig constant $J=9.0$ Hz. The above data and the Mass spectrum which were identical with those of an authentic sample of *p*-hydroxybenzaldehyde have enabled us to propose the structure of VII, as shown in Figure 2. The much higher *p*-hydroxybenzaldehyde content of compression wood compared with other zones seems to be related to the fact that lignin^{8,9)} in compression wood have higher degree of condensed units than that of normal wood, thus *p*-hydroxyphenyl nucleus would have more possibility of condensation in *ortho* positions to phenolic hydroxyl.

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要 約

正常材カラマツは辺心材に、圧縮あてを含む材(辺材)はあて材と側部材に分けて、それぞれの風乾木粉を48時間ずつ3回室温でメタノール抽出し、えられたメタノール抽出物を45°C以下の温度で減圧濃縮した。次いでメタノール抽出物を順次 *n*-ヘキサン、ベンゼン、酢酸エチルで再抽出してそれぞれの可溶部をえた。心材は他の部位に比べて全抽出量が多く、特にベンゼン、酢酸エチル両可溶部において顕著であった。他方、あて材は側部材、辺材より全抽出量が多く、心材の場合と同様にベンゼン、酢酸エチル可溶部が2~3倍に達する。

構成成分を調べるためにシリカゲル・カラムクロマトグラフィをおこなって lignoceryl ferulate (I), pinocembrin (II), naringenin (III), aromadendrin (IV), taxifolin (V), kaempferol (VI) をすべての部位から、*p*-hydroxybenzaldehyde (VII) をあて材からのみ単離した。

オヒヨウニレ、アキニレでは化合物 (I) は、辺材からのみ単離され心材には薄層クロマトグラフィで検出されないことから、心材形成に伴う抽出成分の化学的変化の一つと考えられているが、カラマツではいずれの部位にも同じ程度存在する。

IV, V, VI の化合物がカラマツ (*Larix leptolepis* GORD.) に存在することは確認されているが、その他の化合物の単離は今回が初めてである。III, IV, V の化合物はカラマツの主な抽出成分であるが、特に心材において顕著であった。

他方、圧縮あて材から単離された *p*-hydroxybenzaldehyde (VII) は他の部位にも存在す

るが、量的にかなり少ない。圧縮あて材リグニンは縮合型、非縮合型の *p*-hydroxyphenyl 核に富むことを考慮すると、*p*-hydroxyphenyl 核を有する化合物 (VII) の単離はあて形成となんらかの関係があるように思われる。