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Original

# Studies on Hemicelluloses in Tension Wood

## I. Chemical Composition of Tension, Opposite and Side Woods of Japanese Beech (*Fagus crenata* Blume)*

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**Abstract**——Three different types of woods, tension, opposite and side woods, were isolated from *Fagus crenata* Blume and their anatomical and chemical properties were compared. Tension wood was composed of well developed gelatinous fibers and was characteristic in its low lignin and pentosan contents as well as its high ash, alpha-cellulose and uronic acid contents. Carbohydrate portion of tension wood has been found to be peculiar in that tension wood contains the highest amount of galactose in contrast to the lowest amount of mannose among three types of wood. Each wood was further subjected to sequential fractional extractions. The peculiarity of the chemical composition of the tension wood reflected the differences in the carbohydrate compositions of the extracted hemicelluloses. In analysing the molecular weight distribution of the extracted hemicelluloses by gel filtration on Sepharose 4B, it has been found that all components included in the gel matrices have quite similar molecular weights in spite of the differences in the carbohydrate compositions. These results may give a clue in ellucidation of the distribution and function of hemicelluloses in wood.

### 1. Introduction

Tension wood is formed on the upper side of a leaning or twisted stem and on the upper side of branches in the arborescent angiosperms. Anatomically, typical tension wood fibers differ from normal fibers in containing a cell wall layer, refered to as the gelatinous layer (G-layer)[1] which is less or not lignified and largely consists of cellulose[2~9]. Presence of this G-layer markedly affects the chemical composition of tension wood. The most characteristic chemical feature of tension wood is its low lignin and high cellulose contents. Beside cellulose, tension wood contains more galactose residues than normal wood[10~13]. Timell has reported that tension wood of American beech (*Fagus grandifolia* Ehrl.) contains 6.0% galactan compared with only 1.6% for normal wood[10], and isolated a galactan from this tension wood[14]. The galactan in tension wood is unique among structural wood polysaccharides, both in its complexity and its high degree of branching. Presence of a similar galactan

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** Research Section of Wood Chemistry.

in Europian beech (*Fagus silvatica* L.) has also been proposed by Meier[13,15]. However, information about the chemical structure of hemicelluloses in tension wood is limited to galactan and no characterization has been made on the other molecular species of hemicelluloses. Nothing is also known concerning the distribution of hemicelluloses in tension wood.

In this study, three different types of wood comprising tension, opposite and side woods in the same growth rings were sampled from Japanese beech (*Fagus crenata* Blume), and their chemical properties were compared with special emphasis in the structure of hemicelluloses.

## 2. Experimental

### 2.1 Materials

Japanese beech (*Fagus crenata* Blume), with one bend just above the root was obtained from Kyoto University Forest, Asiu, Miyama-cho, Kyoto. This tree was about 200 years old, and there were several bands of tension wood in the 130–150th growth rings from the pith on the upper side of the bend. These bands gave the characteristic silky luster on the cross surface, providing an identifying mark of tension wood. Tension wood (T), opposite wood (O) and side wood (S) were separately collected as illustrated in Fig. 1. Each wood area was milled to 42–60 mesh in a Willey Mill, and extracted with ethanol-benzene (1 : 2, v/v).

Dextran T fractions (T-10, T-20, T-40, T-70 and T-500), FITC Dextran (FITC-3) and Sepharose 4B were supplied by Pharmacia Fine Chemicals. Extra pure *n*-propionic acid and acetic acid for gas chromatograph were obtained from Gaskuro Kogyo Co., Ltd. All other reagents used were analytical reagent grade.

### 2.2 General methods

Solvents were removed under diminished pressure below 45°C. Total carbohydrate and uronic acid contents in the isolated fractions were determined by the phenol-sulfuric acid method[16], and the modified carbazole method[17], respectively. Klason lignin and acid soluble lignin contents were determined according to the TAPPI standard methods. The lignin content was also determined by the acetyl bromide method[18]. Pentosan, alpha-cellulose, moisture and ash contents were determined by JIS standard methods. Holocellulose content was determined according to the method of Uprichard[19]. The uronic acid anhydride was obtained by the semimicro determination method of Johansson *et al.*[20] Acetyl groups were estimated by g.l.c. on a column (2m×0.3cm) of 20% tetramethylcyclobutanediol adipate-4% phosphoric acid on Chromosorb W at 120°C using *n*-propionic acid as an internal standard[21]. The neutral sugar composition of wood meal was analysed according to Saeman *et al.*[22] The neutral sugar compositions of the isolated fractions

were analysed after hydrolysis with 1N sulfuric acid at 100°C for 6 hr. The hydrolysate was neutralised with barium carbonate and deionised with Dowex 50×8 ($H^+$ form) and Dowex 1×8 (acetate form). The resultant neutral sugars were then converted to corresponding alditol acetates and separated by g.l.c. on a column (2m×0.3cm) of 3% ECNSS-M on Gas Chrom Q at 180°C using methyl β-D-glucopyranoside was used as an internal standard. Configurations of the sugars were determined by g.l.c. on a SP-1000 S.C.O.T. column (25 m×0.28 mm) at 200°C.

G.l.c. for ordinal columns was conducted on a Shimadzu GC-4CM gas chromatograph. G.l.c. for S.C.O.T. column was conducted on a JEOL JGC 1100 gas chromatograph. Both gas chromatograph were equipped with flame-ionisation detectors. Optical rotations were determined with a JASCO DIP-181 digital polarimeter at 20°C. Electron microscopy was performed with a JEM-7 electron microscope (80kV).

### 2.3 Anatomical observation

Small blocks were collected from the fresh tension wood, opposite wood and side wood. Thin sections of about 30 μm thick were cut and stained with safranin-light green or zinc chloride-iodine. Gelatinous layer (G-layer) was clearly detected by using light microscope. For transmission electron microscope, transverse ultrathin sections were prepared from epoxy embedded blocks after block-staining with potassium permanganate.

### 2.4 Fractional extraction

The extracted-free wood meal (50 g) was suspended in water (1 *l*) for 4 hr at 80°C. The extract was recovered by filtration on sintered glass and the solid residue was washed with water (1 *l*). The extract was re-filtered to remove insoluble materials. To this solution 5 volumes of ethanol was added. The solution was kept over night at 5°C, and the precipitated material was recovered by centrifugation. The precipitate was washed with ethanol followed by petroleum ether and dried *in vacuo* at room temperature to give fraction A-1. The solid residue was re-extracted with hot water as described above to give fraction A-2.

The solid residue obtained after hot water treatment was depectinated by treatment with 0.25% potassium acetate (1 *l*) for 24 hr at 60°C. The extract was filtered on sintered glass. The solid residue was washed with water (1 *l*) and acetone successively and dried.

Delignification of the depectinated wood meal was performed according to Klauditz[23]. The depectinated wood meal (100 g) was suspended in 1.0 *l* of hot water warmed at 45°C. To this solution sodium chlorite (80 g) and acetic acid (15 g) were added and the reaction mixture was kept at 45°C for 40 hr with vigorous stirring. The pH of the solution was maintained at 3.8 to 4.0 during reaction. The

delignified wood meal was filtered and the filtrate was immediately neutralised with sodium carbonate and extensively dialysed against tap water. The dialysed solution was concentrated to a small volume and subjected to ethanol precipitation to give fraction B.

The delignified wood meal was suspended in 500 ml of dimethylsulfoxide (DMSO) for 20 hr at ambient temperature. The extract was recovered by filtration on sintered glass, and solid residue was throughly washed with DMSO (100 ml) followed by water (500 ml). The extract and washings were combined and dialysed against tap water to remove DMSO and then concentrated to a small volume and subjected to ethanol precipitation to give fraction C-1. This extraction process was repeated once more to give fraction C-2.

The residual wood meal was subsequently extracted with hot water at 80°C for 4 hr as described above to give fraction D-1. This process was repeated once more to give fraction D-2.

The residual wood meal was further extracted with 10 volumes of 1% potassium hydroxide solution for 4 hr at ambient temperature under nitrogen atmosphere. The extract was recovered by filtration on sintered glass and the solid residue was throughly washed with 1% potassium hydroxide. The extract and washings were neutralised with acetic acid and extensively dialysed against tap water. The dialysed solution was concentrated to a small volume and subjected to ethanol precipitation to give fraction E.

The remained wood meal was finally extracted with 10 volumes of 24% potassium hydroxide solution for 4 hr at ambient temperature under nitrogen atmosphere. The extract was recovered by filtration on sintered glass and the solid residue was washed with 24% potassium hydroxide. The extract and washings were neutralised with acetic acid and exhaustively dialysed against tap water. The dialysed solution was concentrated to a small volume and subjected to ethanol precipitation to give fraction F.

### 2.5 Gel filtration

The molecular weight distribution of the extracted fractions was determined by gel filtration on a column (55.0 × 1.2 cm) of Sepharose 4B at ambient temperature. The gel was equilibrated with 0.025 M sodium phosphate buffer (pH 6.8). Approximately 3 mg of each extracted sample was dissolved in the same buffer and applied to the column. One-ml fractions were collected using a Toyo Model SF 1000 mini fraction collector equipped with a drop counter. The column was calibrated against dextrans of known molecular weight. The molecular weight of each sample was determined from $\bar{V}_e$ *vs.* log $\bar{M}_w$ plot. The sugar content of each fraction was monitered by the phenol-sulfuric acid method[16].

## 3. Results and Discussion

### 3.1 Anatomical properties

Three parts of wood, tension wood (T), opposite wood (O), and side wood (S), were collected as shown in Fig. 1 and were firstly analysed by light microscopy (Fig.2). In tension wood, ground tissue was completely composed of well developed gelatinous fibers which was not stained with safranin (Fig. 2a). This is further clarified by staining with zinc chloride-iodine. The results are shown in Fig. 3. Unlignified G-layer was coloured reddish brown, while lignified secondary wall was coloured yellow. Middle lamelae was considered to be remarkably lignified (Fig. 3a). Side wood seemed to be almost the same with normal wood (Fig. 2c). On the other hand, opposite wood resembled to suppressed wood because of its narrow growth rings and abundance of vessels (Fig. 2b). The secondary wall of the gelatinous fibers consisted of $S_1$, $S_2$ and thick gelatinous layer (G-layer), which was observed as the electron transparent layer in the ultrathin section from embedded block stained with potassium permanganate (Fig. 4). These results confirm those obtained by Saiki *et al.*[24], and are thought to be sufficient to further characterize the chemical composition of these three parts of wood.

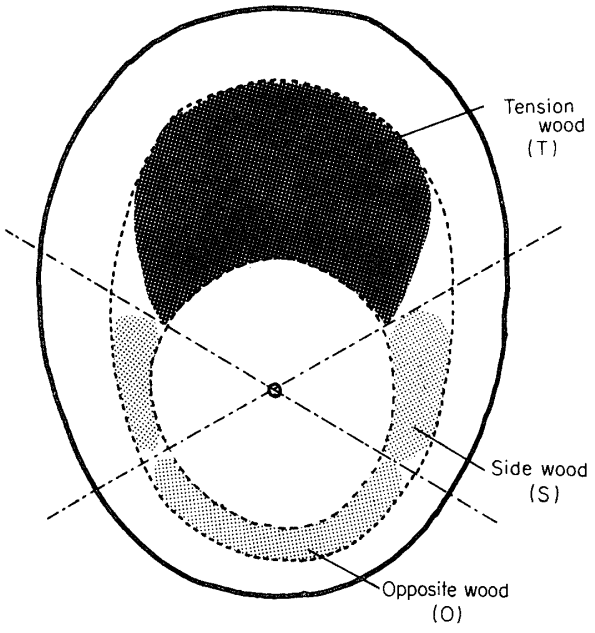



Fig. 1. Schematic illustration of beech wood containing tension wood.

### 3.2 Chemical composition of wood meal

Summative analyses data for tension, opposite, and side woods are listed in Table I. The beech tension wood contained about 8% moisture, which was comparable to about 10% for opposite and side woods. Tension wood contained less lignin and

Table I. Summative chemical composition of tension, opposite and side woods of *Fagus crenata* Blume.*

| Sample | Moisture content | Lignin content | | | Pentosan content | Holocellulose content |
|---|---|---|---|---|---|---|
| | | Klason lignin | Acid soluble | Acetyl bromide | | |
| Tension wood | 7.80 | 19.0 | 3.1 | 18.6 | 17.9 | 81.4 |
| Opposite wood | 10.01 | 20.3 | 3.9 | 19.8 | 21.2 | 80.2 |
| Side wood | 9.53 | 21.3 | 2.9 | 20.3 | 19.8 | 79.4 |
| Sample | Ash content | Uronic acid anhydride | Acetyl content | Alpha-cellulose content | | |
| Tension wood | 0.66 | 5.1 | 2.65 | 50.3 | | |
| Opposite wood | 0.62 | 4.9 | 3.10 | 41.0 | | |
| Side wood | 0.50 | 4.8 | 3.34 | 42.2 | | |
| Sample | Neutral sugar composition** | | | | | |
| | L-Rhamnose | L-Arabinose | D-Xylose | D-Galactose | D-Mannose | D-Glucose |
| Tension wood | 1.1 | 1.7 | 21.8 | 2.3 | 1.5 | 71.7 |
| Opposite wood | 1.0 | 1.1 | 26.2 | 1.5 | 4.8 | 65.5 |
| Side wood | 0.7 | 1.0 | 27.1 | 0.7 | 3.6 | 66.9 |

* All values except neutral sugar composition in per cent are based on extractive free wood meal. ** Values in per cent of neutral sugars.

pentosan and more alpha-cellulose and uronic acid than did the other parts of wood. Ash content in tension wood was also somewhat high. These results were in agreement with the previous results[12,25]. As for the neutral sugar composition, tension wood contained more L-arabinose, D-galactose, D-glucose, and L-rhamnose, and less D-xylose and D-mannose than any other parts of wood. It must be emphasised that tension wood is remarkably high in galactose and low in mannose. These results are in line with those obtained by Timell[10]. He concluded that the $S_1$ and $S_2$ layers in a gelatinous fiber contained less glucomannan and more galactan than did normal fibers. Assuming that the chemical structure of xylan in tension wood is the same as that of xylan in normal wood, consisting of partially acetylated $\beta(1\rightarrow4)$ linked D-xylopyranose residues having a small amount of 4-*O*-methylglucuronic acid as side chains, tension wood must contain less acetyl and uronic acid than normal wood. As for acetyl content, present results support this posturation. A similar result has been reported by Schwerin for *Eucalyptus goniocalyx*[12]. In contrast to acetyl content, uronic acid content in the present investigation did not coincident with the posturation. This may be, however, due to the presence of the similar highly acidic galactan in tension wood as that isolated by Timell[10]. Comparison of the results for opposite and side woods reveals that no substantial difference in the lignin and neutral sugar composition. Arabinose and rhamnose residues which are rich in tension wood may

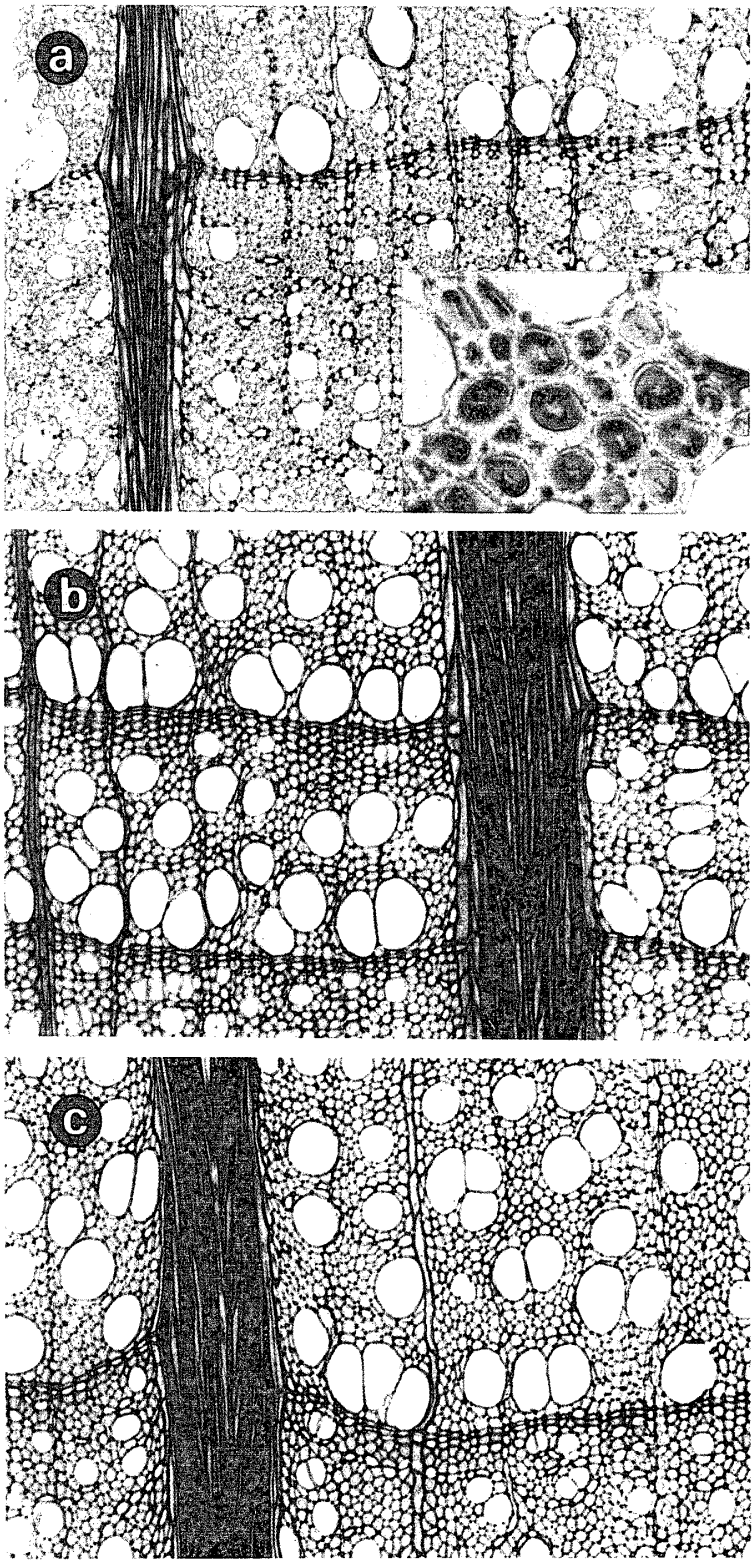
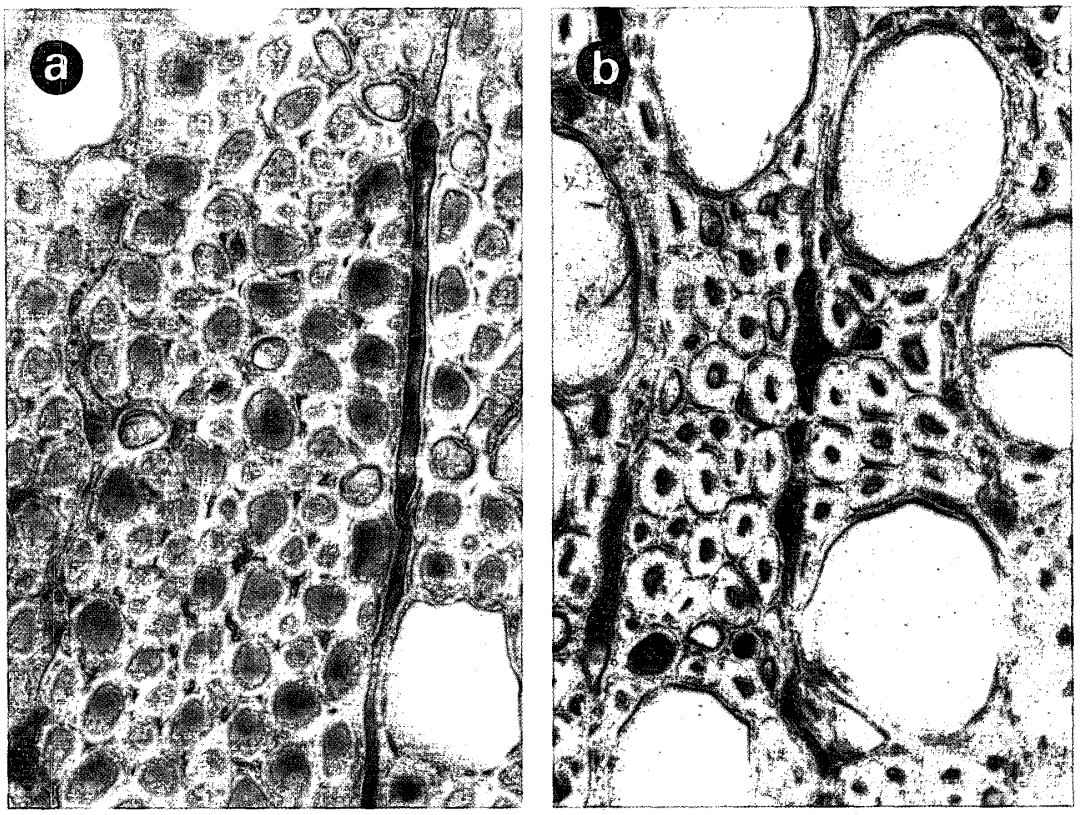



Fig. 2. The transverse section of tension wood (a), opposite wood (b), and side wood (c) stained with safranin-light green (×33). Inset shows the transverse section of tension wood at higher magnification (×133).




(a) (b)

Fig. 3. The transverse section of tension wood (a) and opposite wood (b), stained with zinc chloride-iodine (×133).

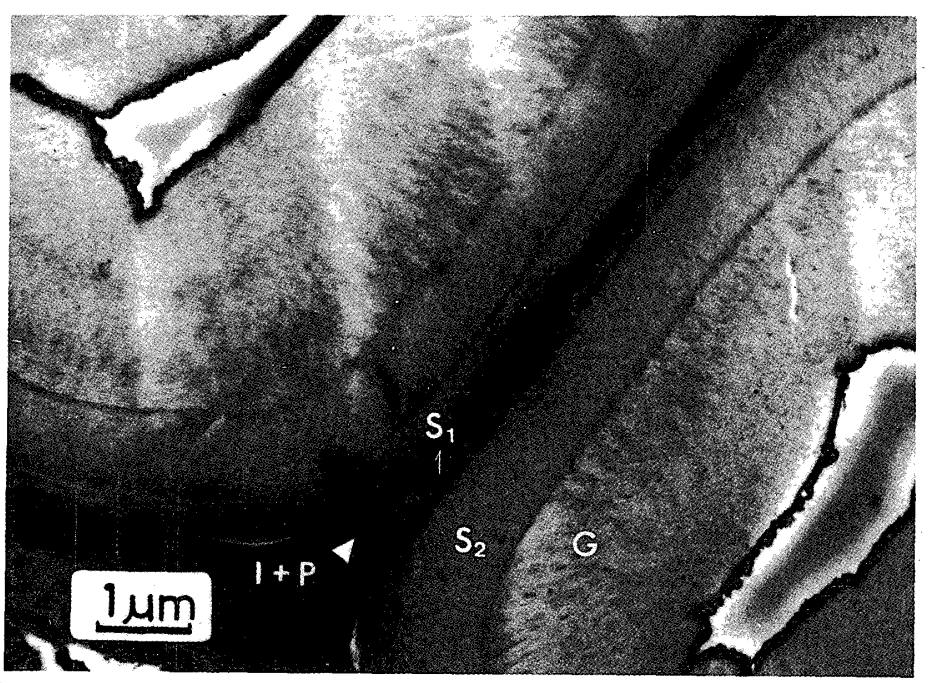



Fig. 4. Gelatinous fiber wall in tension wood. I: Intercellular layer, P: Primary wall, $S_1$: Outer layer of the secondary wall, $S_2$: Middle layer of the secondary wall, G: Gelatinous layer.

be due to pectin as is assumed by Timell[10]. The chemical composition of side wood is in quite agreement with that of normal wood, confirming the anatomical results.

### 3.3 Chemical composition of extracted fractions

Tension, opposite and side woods were individually extracted with water (fraction A) and delignified (fraction B). The hollocellulose thus obtained was depectinated and subjected to sequential fractional extractions to give fractions C to F. The yields and chemical properties of these extracts are summarised in Tables II–IV. The fraction A is peculiar in its high content of galactose and glucose, and low content of xylose. Although the origin of glucose in this fraction is obscure, galactose residues may be partly due to pectin. This pectin like polysaccharide may also be present in the fraction B recovered from the chlorite liquor, since Meier has reported that tension wood galactan could not be dissolved at all by delignification[13]. Meier has further reported that stepwise extraction of the hollocellulose with DMSO and hot water yields almost no galactose containing polysaccharides and that a larger

Table II. Chemical composition of the extracted fractions of tension wood.

| Fraction | Neutral sugar composition* | | | | | |
|---|---|---|---|---|---|---|
| | L-Rhanmose | L-Arabinose | D-Xylose | D-Galactose | D-Mannose | D-Glucose |
| A-1 | 2.7 | 7.2 | 1.0 | 13.2 | t | 75.8 |
| A-2 | 8.7 | 11.7 | 1.0 | 37.0 | 1.4 | 40.2 |
| B | 9.3 | 17.9 | 26.3 | 29.8 | t | 16.7 |
| C-1 | 0.0 | 1.9 | 98.1 | t | t | t |
| C-2 | 1.3 | 1.4 | 55.5 | 1.6 | 4.2 | 36.0 |
| D-1 | 5.2 | 9.8 | 57.3 | 23.9 | t | 3.8 |
| D-2 | 2.7 | 2.9 | 80.0 | 7.7 | t | 6.8 |
| E | 5.0 | t | 81.1 | 11.2 | 2.7 | 0.0 |
| F | 1.2 | t | 97.9 | 0.0 | 0.9 | t |
| Fraction | Yield** | Uronic acid content*** | Acetyl content*** | $[\alpha]_D^{20}$ | | |
| A-1 | 0.94 | 14.0 | 1.08 | +72.4° | | |
| A-2 | 0.23 | 12.0 | 1.60 | +56.3° | | |
| B | 4.30 | 7.6 | 2.89 | +14.3° | | |
| C-1 | 2.92 | 4.8 | 6.27 | −41.5° | | |
| C-2 | 0.87 | 5.2 | 3.92 | −15.2° | | |
| D-1 | 1.87 | 14.0 | 4.51 | −17.7° | | |
| D-2 | 1.36 | 12.8 | 5.50 | −15.1° | | |
| E | 5.94 | 11.2 | — | −29.8° | | |
| F | 2.95 | 10.8 | — | −72.1° | | |

* Values in per cent of neutral sugars. ** Values in per cent of extractive free wood.
*** Values in per cent of extracted materials. t Trace.

Table III. Chemical composition of the extracted fractions of opposite wood.

| Fraction | Neutral sugar composition* | | | | | |
|---|---|---|---|---|---|---|
| | L-Rhamnose | L-Arabinose | D-Xylose | D-Galactose | D-Mannose | D-Glucose |
| A-1 | 1.4 | 9.8 | 1.4 | 8.6 | 9.3 | 69.4 |
| A-2 | 1.1 | 4.2 | 1.3 | 3.7 | 7.6 | 82.2 |
| B | 2.4 | 12.0 | 41.3 | 5.9 | 4.0 | 34.4 |
| C-1 | t | 2.0 | 86.0 | 2.9 | 4.4 | 4.6 |
| C-2 | 3.2 | t | 60.2 | 4.1 | 5.4 | 27.1 |
| D-1 | 3.1 | 13.9 | 52.4 | 9.2 | 10.0 | 11.4 |
| D-2 | t | 1.5 | 87.3 | 4.1 | 3.0 | 4.1 |
| E | 0.0 | 1.8 | 98.2 | t | t | t |
| F | 0.0 | 1.2 | 98.7 | t | t | t |

| Fraction | Yield** | Uronic acid content*** | Acetyl content*** | $[\alpha]_D^{20}$ |
|---|---|---|---|---|
| A-1 | 0.75 | 2.4 | 1.92 | +65.7° |
| A-2 | 0.08 | 5.2 | 2.55 | +79.4° |
| B | 2.87 | 7.2 | 3.13 | +19.2° |
| C-1 | 2.05 | 4.4 | 5.64 | −30.9° |
| C-2 | 0.58 | 3.8 | 3.06 | −18.7° |
| D-1 | 3.84 | 13.2 | 4.36 | −13.1° |
| D-2 | 1.73 | 12.2 | 6.73 | − 1.5° |
| E | 7.56 | 10.0 | — | −43.8° |
| F | 3.32 | 7.1 | — | −80.9° |

* Values in percent of neutral sugars. ** Values in per cent of extractive free wood.
*** Values in per cent of extracted materials. t Trace.

amount of galactan can be extracted with 8% sodium hydroxide accompanied by xylan[13]. Timell has also isolated galactan from fully depectinated and delignified wood meal by extraction with 10% sodium carbonate[10]. Present results indicate that contents of galactose in fractions D and E are not so high as the results of Meier[13]. Although the reason of this discrepancy is not clarified at present, it may be expected that the fraction D-1 is used to obtain galactan from tension wood. Fraction C-1 is entirely xylan with acetyl. Therefore this fraction could be used to obtain native acetylated xylan from wood. While, fractions E and F extracted with potassium hydroxide are attractive source for preparation of xylan devoid of acetyl. Since no structural analysis of tension wood xylan has been performed, studies on this direction are now in progress.

### 3.4 Gel filtration analysis

Molecular weight distributions of the extracted fractions were analysed by gel filtration on Sepharose 4B gel column (Figs. 5–10). Fractions A-2 and D-2 gave

Table IV. Chemical composition of the extracted fractions of side wood.

| Fraction | Neutral sugar composition* | | | | | |
|---|---|---|---|---|---|---|
| | L-Rhamnose | L-Arabinose | D-Xylose | D-Galactose | D-Mannose | D-Glucose |
| A-1 | 1.7 | 8.4 | 1.6 | 7.3 | 6.0 | 75.1 |
| A-2 | t | 7.7 | 1.5 | 6.4 | 9.3 | 75.0 |
| B | t | 16.3 | 31.0 | 17.9 | t | 34.8 |
| C-1 | 7.6 | 4.3 | 60.0 | 7.4 | t | 20.6 |
| C-2 | 3.9 | 3.5 | 57.6 | 2.7 | 3.9 | 28.3 |
| D-1 | 3.9 | 9.4 | 34.7 | t | 29.7 | 22.4 |
| D-2 | 0.0 | 19.6 | 61.4 | 0.0 | 10.0 | 9.0 |
| E | t | t | 100.0 | t | t | t |
| F | 1.5 | t | 97.5 | t | 1.0 | t |

| Fraction | Yield** | Uronic acid content*** | Acetyl content*** | $[\alpha]_D^{20}$ |
|---|---|---|---|---|
| A-1 | 0.72 | 3.6 | 1.34 | +66.4° |
| A-2 | 0.57 | 4.8 | 1.90 | +69.9° |
| B | 4.02 | 2.4 | 2.70 | − 1.8° |
| C-1 | 1.78 | 3.6 | 0.58 | −90.5° |
| C-2 | 0.39 | 3.2 | 2.87 | −83.3° |
| D-1 | 0.97 | 6.8 | 4.27 | − 7.5° |
| D-2 | 1.15 | 9.6 | 4.64 | − 4.9° |
| E | 9.92 | 9.2 | — | −24.5° |
| F | 4.83 | 6.4 | — | −67.8° |

* Values in per cent of neutral sugars. ** Values in per cent of extractive free wood.
*** Values in per cent of extracted materials. t Trace.

quite similar elution profiles to corresponding A-1 and D-1 fractions, respectively. In C-series, all fractions except C-1 fractions from tension and opposite woods were extremely difficult to dissolve in 0.025M sodium phosphate buffer and showed no distinct peaks on gel filtration. The column was calibrated against dextrans of known molecular weight. All fractions were separated into two major peaks, the one was revealed at the void volume, and the other was included in the gel matrices and eluted at weight average molecular weight of 22,300–26,300. The fundamental difference among the elution profiles is in the variation of the ratio of the amounts of these two peaks. These results indicate that three parts of wood (tension, opposite and side woods) are composed of hemicelluloses having similar molecular weight.

In conclusion, although tension wood is peculiar in its high content of galactan, isolation of this galactan from tension wood is much difficult than that of xylan. Since the fraction D-1, hot water extract after DMSO extraction, contains considerable amount of galactan, this fraction may be used to isolate galactan in tension wood of *Fagus crenata* Blume.

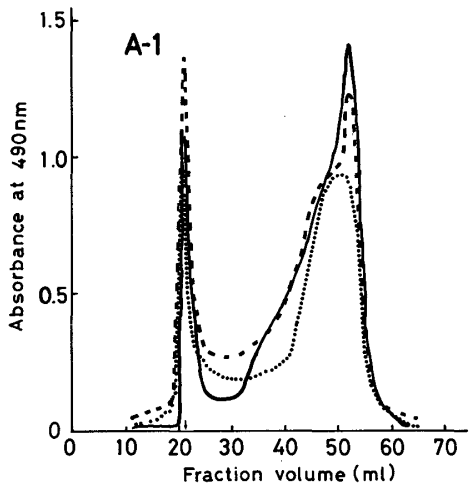



Fig. 5. Gel filtration of the A-1 fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), opposite wood (---), and side wood (···).

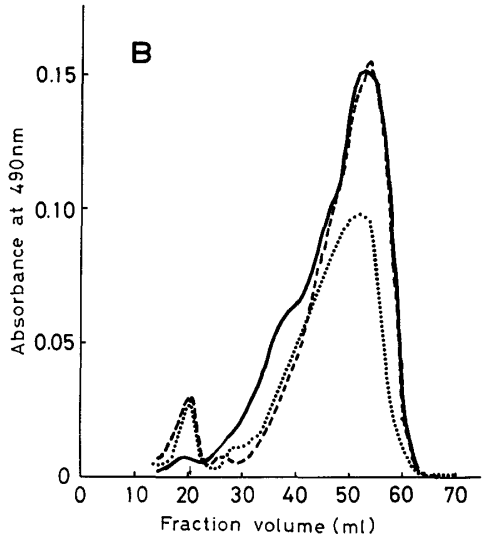



Fig. 6. Gel filtration of the B fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), opposite wood (---), and side wood (···).

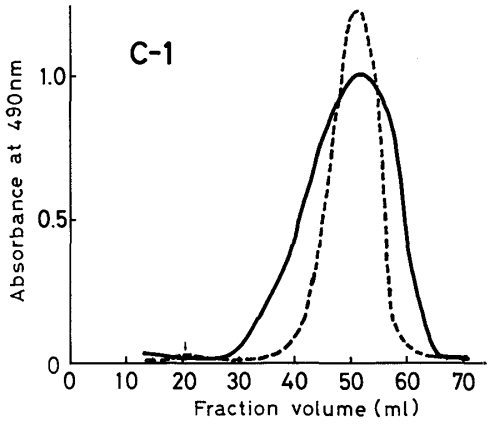



Fig. 7. Gel filtration of the C-1 fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), and opposite wood (---).

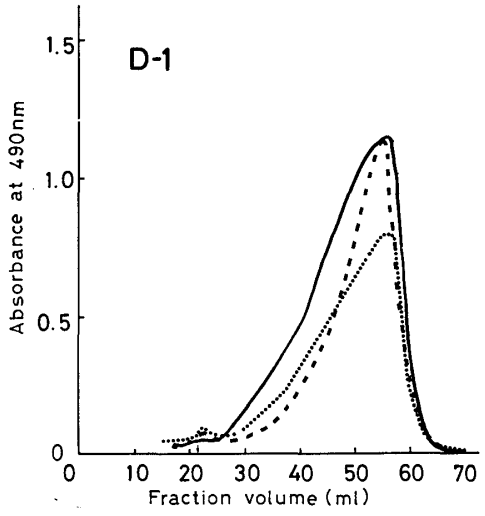



Fig. 8. Gel filtration of the D-1 fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), opposite wood (---), and side wood (···).

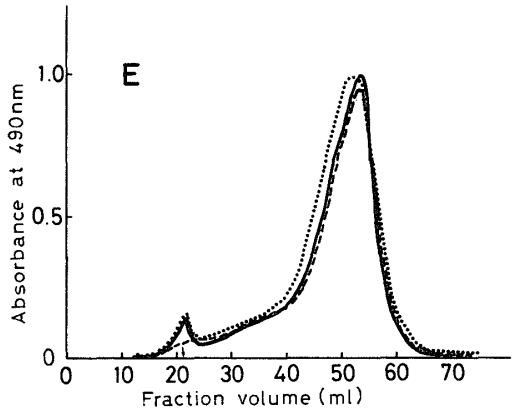



Fig. 9. Gel filtration of the E fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), opposite wood (---), and side wood (···).

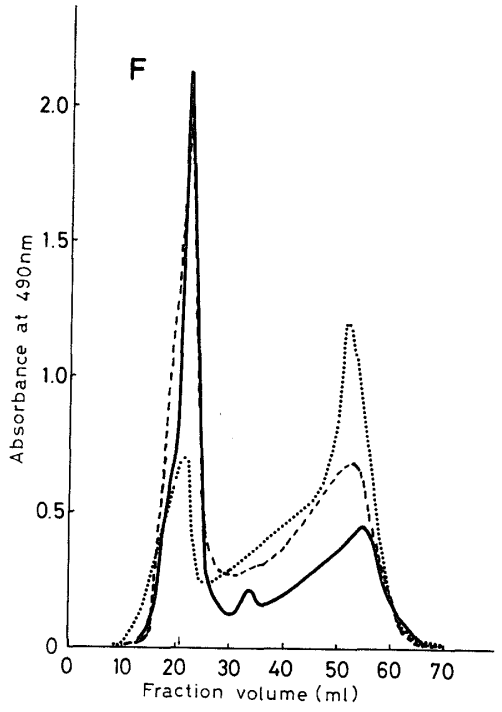



Fig. 10. Gel filtration of the F fraction on Sepharose 4B. The arrow represents the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (—), opposite wood (---), and side wood (···).

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