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Studies on the Extractives of Larch

Report 11*: Determination of Flavonoids in *Larix leptolepis*

By

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カラマツの抽出成分に関する研究

第11報 カラマツ (*Larix leptolepis*) のフラボツノイドの定量

笹谷宜志**・出町秀也***・寺沢 実****

CONTENTS

Introduction

In the previous paper of this series[1)], the analysis of phenolic compounds from the heartwood was described. Two flavonols and two flavanonols were isolated,

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* Report 1 is "Analysis of Phenolic Compounds from the Heartwood of *Larix leptolepis*" in the series "Studies on the Extractives of Larch"., Proc. Hokkaido Branch Japan Wood Res. Soc., No. 1, 57 (1968).

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and these were identified as taxifolin (dihydroquercetin: 3, 5, 7, 3′, 4′-pentahydroxy flavanone), aromadendrin (dihydrokaempherol: 3, 5, 7, 4′-tetrahydroxy flavanone) quercetin (3, 5, 7, 3′, 4′-pentahydroxy flavone) and kaempherol (3, 5, 7, 4′-tetrahydroxy flavone), respectively. Information that kaempherol can be isolated from heartwood of *Larix leptolepis* as a crystal is not yet available although keampherol was found in extracts from heartwood of *Larix* sp. by means of paper partition chromatography[2).

It is well known that one of these flavonoids taxifolin is an anti-pulping agent for the calcium-base sulphite process[3),4). The heartwood of *Larix* sp. is less susceptible to attack by wood-destroying organisms than the sapwood. The fungicidal properties of *Larix* sp. heartwood are similar to Hiba *Thujopsis dolabrata* SIEB, et ZUCC., Hinoki *Chamaecyparis obtusa* SIEB. et. ZUCC. and Kuri *Castanea crenata* SIEB et ZUCC. These species are recognized as durable trees. The durability of a specific heartwood might be due to toxic material in the heartwood. Experimental research in this field has been done by a number of investigators. NISHIDA and co-workers[5),6) have demonstrated that wood phenolics distylin (taxifolin) is resistant to the attack of white rot *Polysticus sanguineus* (L) FRIES. and brown rot *Poria vaporaria* FERS. Also taxifolin is the dominant factor in Douglas-fir durability[7). Taxifolin is also readily converted to quercetin and possesses antioxidant activity preventing rancidity in fat oils and dairy products[8). The oxidation product of taxifolin, quercetin, also demonstrates effective antioxidant properties and, with some substrates, has greater antioxidant activity than taxifolin. The constituents of heartwood have several properties that effect utilization of the wood.

As a factor in the elucidation of the mechanism of heartwood formation, it is very important to determine the content of flavonoids in the wood of *Larix leptolepis* and also to understand the general pattern of variation in concentration within the stem.

It has been reported that the concentration of flavonoids in the wood of the western larch *Larix occidentalis* is greatest at the sapwood-heartwood boundary. Within the tree, a general pattern of decreasing concentration with increasing distance from this boundary to the pith and bark was evident in the cross-section of the western larch[9),10).

The present paper reports the determination of the flavonoid content in the cross-section of the stem of *Larix leptolepis* using spectrophotometric methods in order to discover the pattern of flavonoid variation within the stem.

Experimental

1. Starting Materials and Sampling Procedures

In this investigation, the raw material used was the same sample used in Report 1 of the series. The tree was felled in Tomakomai College Experimental Forest, Hokkaido University, in October, 1967. A disk was collected from the tree trunk 1.3 meters (breast height) from the ground level. The tree had 38 annual rings at breast height and was about 30 centimeters in diameter. The sapwood

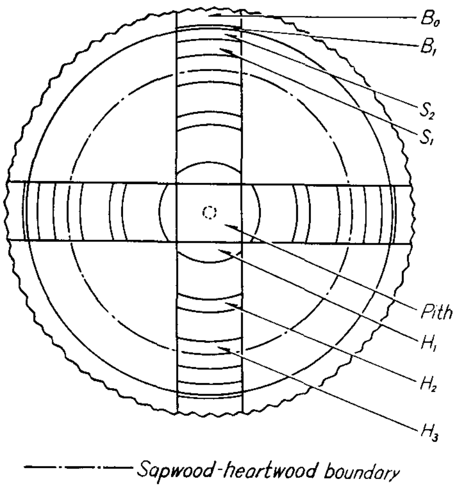

Fig. 1. Sampling mode.

was wide, averaging about 2.7 centimeters, and the sapwood-heartwood boundary was well delineated. A strip 4 centimeters in width was taken from bark to pith along the radius of the disk. As shown in Fig. 1, a strip was divided into 8 sections, i.e. the bark was divied into 2 parts outer bark (B_0) and inner bark (B_i), sapwood into 2 parts (S_1 and S_2), heartwood into 3 parts (H_1, H_2, and H_3) and the pith area was not divided. Each section was allowed to air-dry and then shaved. The shavings of each section were reduced to dust in a Wiley mill until the meal of both bark and wood passed through a 40 mesh screen. The samples obtained were stored in polyethylene bags. The moisture contents of air-dried samples of wood and bark were 7.3% and 8.8%, respectively.

2. Extraction Procedures and Stock Solutions

One gram sample of air-dried dust was placed in a 100 mℓ Erlenmeyer flask with 40 mℓ of methyl alcohol. The flask was fitted with a condensor and heatde on a steam bath for 3 hours. Then the methyl alcohol was decanted.

It is well known that flavonoids are reduced to the corresponding cyanidin chloride by metal (magnesium or zinc)-hydrochloric acid reagents. In the Mg–HCl test, flavones, flavonols, flavanones and flavanonols are colored orange to purple red, but chalcones and isofavones are negative. On the other hand, chalcones, flavones, flavonols and isoflavones are negative in Zn–HCl test, but flavanones and flavanonols are positive.

The decanted alcohol solution was treated with Mg–HCl and Zn–HCl. Methyl alcohol extraction was continued until the alcohol solution showed a negative reaction for flavanones. After the third extraction, extraction was done with 20 mℓ of methyl alcohol added to each sample. Finally, this procedure was repeated 4 times for pith, H_1, H_2 and H_3, 3 times for S_1 and S_2, 10 times for B_i and B_0. Each residue was washed with a small amount of methyl alcohol and the washings were combined with the extracted alcohol solution. The total volume of these alcohol solutions, as stock solutions, was 150 mℓ for pith, H_1, H_2 and H_3; 100 mℓ for S_1 and S_2; 200 mℓ for B_i; and 240 mℓ for B_0, respectively. Each stock solutions for determination of the flavonoid content was kept at room temperature.

3. Ultra-violet Absorption Spectra of Flavonoids

Authentic specimens of taxifolin, aromadendrin, quercetin and kaempherol were dissolved in a 25 mℓ volumetric flask with methyl alcohol and 10^{-4} M solutions of each compound were prepared for scanning. Ultra-violet absorption spectra scanned in neutral medium and alkaline medium using a 1N aqueous solution of sodium carbonate were obtained with a Hitachi EPS–3T Spectrophotometer. These results are shown in Figures 2, 3, 4, and 5.

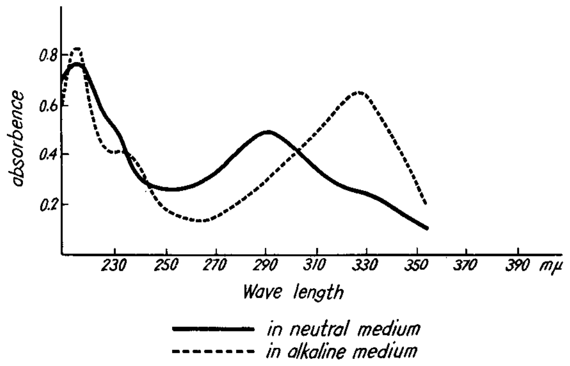

Fig. 2. Ultra-violet absorption spectra of taxifolin.

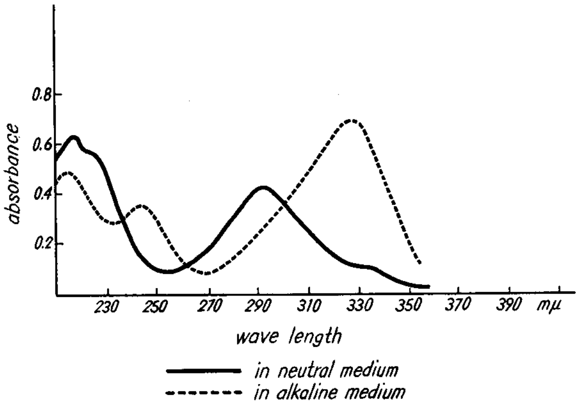

Fig. 3. Ultra-violet absorption spectra of aromadendrin.

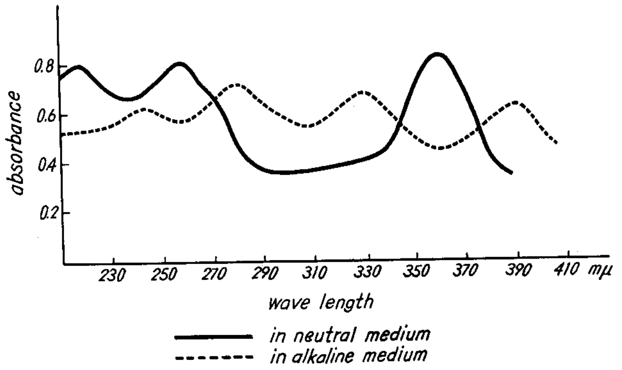

Fig. 4. Ultra-volet absorption spectra of quercetin.

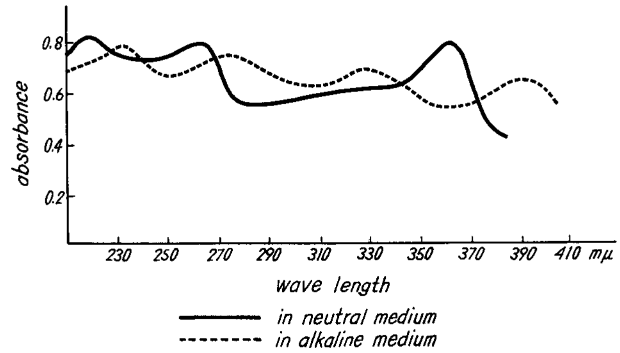

Fig. 5. Ultra-violet absorption spectra of kaempherol.

4. Calibration Curve for Determination of Flavonoids

It has already been reported that taxifolin in woody material is quantitatively determined with precision using the method of absorption photometry with visible[9),10)] and ultro-violet light[11),12)]. In this work, flavonoids were determined by the method developed by KONDO and co-worker[11)]. From the results of the ultra-violet absorption spectra (experiment 3), it is difficult to determine the content of the four compounds by each calibration curve. Therefore, the content of taxifolin and aromadendrin was measured as flavanonols and that of quercetin and kaempherol as flavonols. The content of flavanonols and flavonols was calculated with the calbration curve of taxifolin and quercetin, respectively.

An authentic specimen of 0.695 mgm. of taxifolin was dissolved in a 25 mℓ volumetric flask with methyl alcohol and a 10^{-4} M stock solution of taxifolin was prepared for the determination of the calibration curve. This stock solution was diluted with methyl alcohol to produce solutions one-half, -third, -fourth, -sixth and -eighth the concentration of the stock solution. The absorbances of these six solutions at 294 mμ were plotted and the correlation between absorbance and concentration of the solution obtained. These results are shown in Table 1 and in Figure 6. After the measurement in neutral medium, a small amount of 1N sodium carbonate solution was added in a sample cell and the absorbance in alkaline medium at 326 mμ was mearsred. A few drops of alkali was sufficient for this purpose.

Table 1. Correlation between Absorbance and Concentration in diluted Solution of Taxifolin.

A multiple of dilution	Concentration (10^{-4} mol)	Absorbance in neutral medium (294 mμ)	Absorbance in alkaline medium (326 mμ)
1	0.9144	1.30	1.80
2	0.4572	0.595	0.940
3	0.3048	0.431	0.570
4	0.2286	0.305	0.445
6	0.1524	0.233	0.284
8	0.1143	0.156	0.220

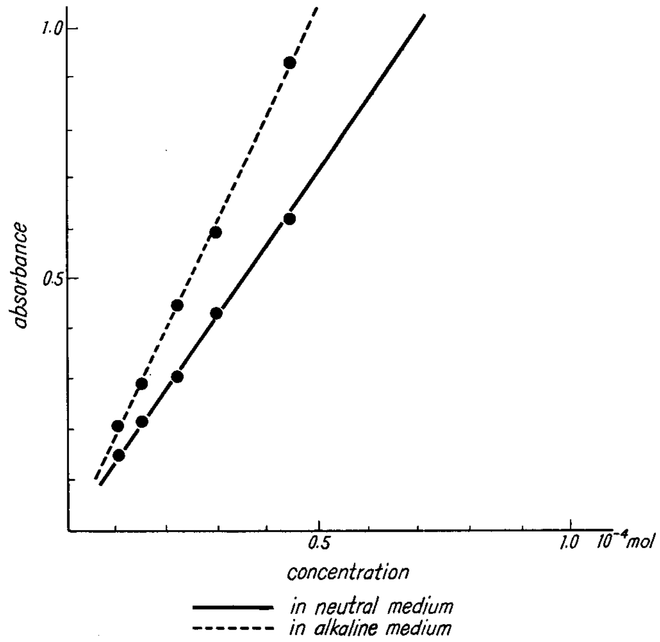

Fig. 6. Calibration curve of taxifolin.

The pH range was 8.4–8.6. A precipitate was not produced in the cell when an alkali solution was added. Similarly, the same portion of alkali solution was added to a reference cell and the absorbance was measured after 15 minutes.

For the measurement of quercetin, 1.114 mgm. of authetic specimen was dissolved in a 25 mℓ volumetric flask with methyl alcohol and this stock solution was diluted to one-tird, -fourth, -fith, -sixth, -eighth and -tenth. The absorbance of each quercetin solution was measured at 360 mμ for neutral solutions and at 392 mμ for alkaline solutions. These results are shown in Table 2 and Figure 7. A Hitachi EPU-2A Spectrophotometer was used.

Table 2. Correlation between Absorbance and concentration in diluted Solution of Quercetin.

A multiple of dilution	Concentration (10^{-4} mol)	Absorbance	
		in neutral medium (360 mμ)	in alkaline medium (392 mμ)
3	0.4918	1.06	0.541
4	0.3689	0.792	0.410
5	0.2951	0.638	0.328
6	0.2459	0.514	0.279
8	0.1844	0.389	0.199
10	0.1476	0.302	0.155

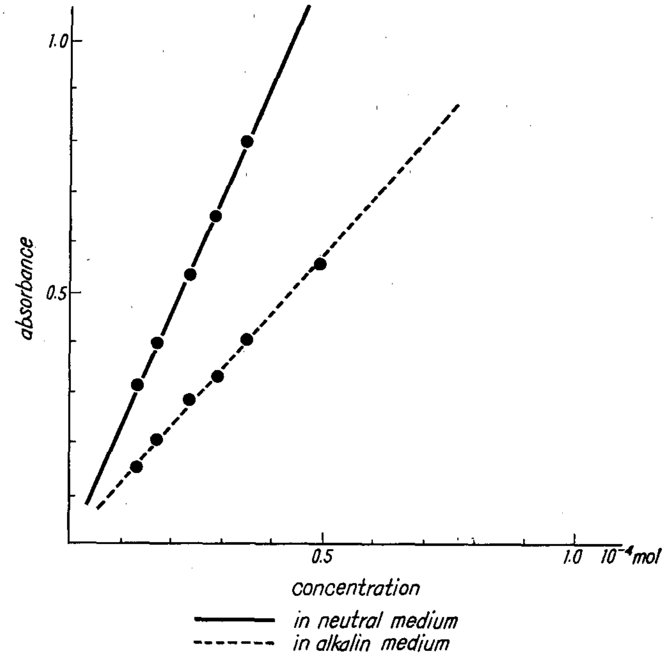

Fig. 7. Calibration curve of quercetin.

5. Determination of Flavonoids in the Extracts

To determine flavanonol as taxifolin, the extracted stock solution was diluted suitably in order to produce an absorbance in a convenient range and was measured in neutral medium at 294 mμ. The concentration of flavanonol was obtained from the curve correlating the measured absorbance and concentration. Then the flavanonol content in raw material was calculated by substituting the flavanonol concentration into the the following equation (1).

$$X(\%) = \frac{n.a.M.v}{W.1000} \times 100 \qquad (1)$$

X: content to oven-dried raw material
n: a multiiple of dilution
a: concentration of flanonoid in the diluted solution
v: volume of extracted stock solution
M: molecular weight of authentic specimen
W: weight of over dried raw material

The flavonol content as quercetin was measured at 360 mμ in the same way as flavanonol. Also the determination of the content of flavanonol and flavonol in alkaline medium at 326 mμ and at 392 mμ was carried out in the same way as the determination in neutral medium.

These results are shown in Table 3 and 4, and in Figures 8 and 9.

Table 3. The Content of Flavanonol as Taxifolin.

Each section	A multiple of dilution (mℓ)	Absorbance of diluted solution		Concentration of diluted solution (10^{-4} mol)		Content in raw material (%)	
		in neutral medium	in alkaline medium	in neutral	in alkaline	in neutral	in alkaline
Pith	150×24	0.476	0.547	0.34336	0.28034	3.92	3.20
H_1	150×24	0.560	0.680	0.40266	0.34700	4.69	4.04
H_2	150×24	0.700	0.930	0.50150	0.47227	5.86	5.52
H_3	150×24	0.813	0.970	0.58127	0.49231	6.87	5.82
S_1	100×24	0.205	0.170	0.15204	0.09147	1.68	0.71
S_2	100×24	0.102	0.059	0.07933	0.03585	0.80	0.28
B_i	200×24	0.622	0.367	0.44643	0.19017	7.06	3.01
B_0	240×24	0.469	0.176	0.33842	0.09447	6.47	1.81

Table 4. The Content of Flavonol as Quercetin.

Each section	A multiple of dilution (mℓ)	Absorbance of diluted solution		Concentration of diluted solution (10^{-4} mol)		Content in raw material (%)	
		in neutral	in alkaline	in neutral	in alkaline	in neutral	in alkaline
Pith	150×1	0.800	0.478	0.37263	0.43128	0.176	0.204
H_1	150×1	0.723	0.508	0.33765	0.45808	0.163	0.221
H_2	150×1	0.900	0.565	0.41806	0.50900	0.202	0.246
H_3	150×1	0.945	0.599	0.43851	0.53937	0.215	0.262
S_1	100×1	0.302	0.258	0.14637	0.23476	0.047	0.076
S_2	100×1	0.262	0.202	0.12820	0.18473	0.042	0.060
B_i	200×4	0.462	0.686	0.21906	0.61709	0.574	1.616
B_0	240×4	0.202	0.315	0.10094	0.28568	0.319	0.904

Results and Discussion

The ultra-violet absorption spectra of four flavonoids are shown in Figures 2, 3, 4 and 5. Taxifolin and aromadendrin in neutral meeium have an absorption maximum at 294 mμ which is characteristic of flavanones. In alkaline medium, this absorption maximum is shifted to 326 mμ. On the other hand, quercetin and kaempherol show an absorption maximum at 360 mu in neutral solution and an absorption maximum at 392 mu in alkaline solution. As mentioned above, the taxifolin absorption curve is almost identical to the aromadendrin absorption curve and the querectin absorption curve is almost identical to the kaempherol curve. The calibration curve of taxifolin is, therefore, employed for the measurement of flavanonol content and that of quercetin is employed for the measurent of flavonol content.

The stock solution (10^{-4} M) of taxifolin is diluted and six solutions of the different concentrations are prepared. The results of these measurements are shown in Table 1. The measurement of quercetin is done in a similar way and those results are tabulated in Table 2. The correlation between absorbance and concentration is shown in Figures 6 and 7. There is an obvious rectilinear relationship between absorbance and concentration in these figures. The results with these diluted solutions evidently satisfy Lambert-Beer's correlation. When the concentration of each solution is calculated from the graphs, there are unavailable errors introduced. In our measurement, the determination of the content of flavonoids in several solutions is computed by the equation which was derived from the methods of least squares.

The equations of each specimen in neutral and alkaline nedium are as follows:

Taxifolin

in neutral : $Y = 1.4165\,x - 0.01037$ (2)

in alkaline : $Y = 1.9958\,x - 0.01255$ (3)

Quercetin

in neutral : $Y = 2.2010x - 0.02016$ (4)

in alkaline : $Y = 1.1195x - 0.0048$ (5)

Y: Absorbance ($-\log T$)

x: Concentration of dilute solution (10^{-4} M)

The diluted solution of the extracted stock solution from each section was analysed and the concentration was calculated by the above equation (2, 3, 4 and 5). Then, the content (%) of flavonoids in the raw material was calculated using equation 1. There results are shown in Table 3 and 4.

As mentioned before, the flavonoid content is considered to be the sum of taxifolin and aromadendrin even though other compounds may have contributed to the measured absorbance. However, from the results in the previous report (1), taxifolin and aromadendrin are the major flavanonol components and taxifolin is the dominant constituent in methyl alcohol extracts. Likewise, the flavonol, quercetin, is the main compound in the flavonol group. Although the measured values do not indicate the exact content of taxifolin and querecetin, the measured values are satisfactory to study the variation of flavonoids within the stem.

As the shown in Fig. 8 which shows the determinations in neutral medium, the content of flavanonol within the disk gradually increases from pith to the sapwood-heartwood boundary. The values are pith$=$3.92%$\rightarrow H_1=$4.69%$\rightarrow H_2=$5.86%$\rightarrow H_3-$6.87%, respectively, On the other hand, the values decrease from the sapwood-heartwood boundary to the cambial zone. The flavanonol content of S_1 and S_2 are 1.68% and 0.80%. Although bark is anatomically different from wood, the content of flavanonol in bark is quite high, though it may be not related to their distritubion directly. Flavanonol contents are 7.06% in B_i and 6.47 in B_0.

In alkaline medium, the contents are pith$=$3.20%$\rightarrow H_1=$4.04%$\rightarrow H_2=$5.52%$\rightarrow H_3=$5.82%$\rightarrow S_1=$0.71%$\rightarrow S_2=$0.28% and $B_i=$3.01% and $B_0=$1.81%. These values

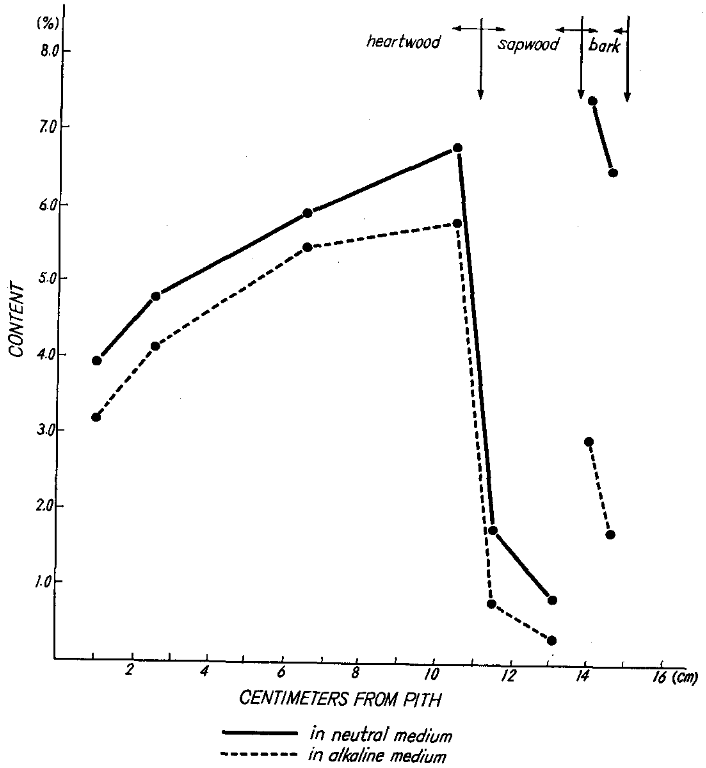

Fig. 8. Distribution of flavanonol (as Taxifolin) of *Larix leptolepis.*

are lower than those of the neutral method. Flavanonol contents in the bark are considerably reduced by alkali treatment.

It is interesing that the variation in flavanonol content shows similar tendencies in both neutral and alkaline methods. The higher values in neutral medium seem to be due to co-existance of unidentified compounds other than taxifolin and aromadendrin, or alternatively, the values in neutral medium may affect the absorbance of flavonol contained in the extracted stock solution at 294 mμ in some way. As is generally known, the absorption maxinum of many phenolic compounds is shifted to the visible region in alkaline solutions. The maximum of taxifolin is transfered to the longer wave length region with alkali and occurs at 326 mμ. This bathochromic behaviour of phenolics is a characteristic of each compound. When the stock solution is treated with alkali, the absorption maxima of some compounds are expected to be at wave lengths other than 326 mμ. As mentioned above, the flavanonol contents of bark measured at 326 mμ are remarkedly decreased. Althought is can not be definitely concluded, it is assumed that the bark extracts contain mainly substances other than flavanonols, whose absorption maxima in alkaline medium are transfered to another wave length region outside 326 mμ.

Considering the accuracy of the measurements, it is more suitable for our purpose that the measurement in alkaline medium is practiced with more precision than that in neutral medium because the alkaline medium appears to remove the

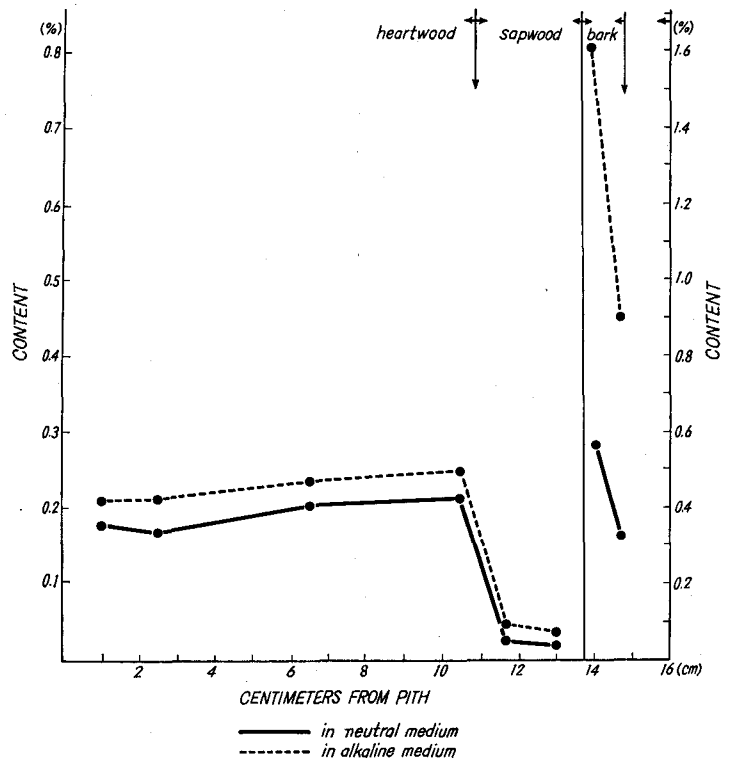

Fig. 9. Distribution of flavonol (as Quercetin) of *Larix leptolepis*.

interference to flavanonol measurement.

The distribution pattern of flavonol as quercetin in the disk of larch is shown in Fig. 9. Using the neutral method, the contents are pith$=0.176\%\rightarrow H_1=0.163\%\rightarrow H_2=0.202\%\rightarrow H_3=0.215\%$ in the heartwood. The flavonol content increases slightly from pith to the sapwood-heartwood boundary, and decreases from this boundary to the cambial zone. The flavonol content decreases considerably like the variation of flavanonol. The results are $S_1=0.047\%$ and $S_2=0.042\%$. Within the bark, flavonol contents are $B_i=0.574\%$ and $B_0=0.319\%$. When the contents are measured in alkaline medium, the contents of flavonol are as follows: pith$=0.204\%\rightarrow H_1=0.221\%\rightarrow H_2=0.246\%\rightarrow H_3=0.262\%\rightarrow S_1=0.076\%\rightarrow S_2=0.060\%$ and $B_i=1.616\%$ and $B_0=0.904\%$. The content of flavonol in the disk is also increased at the sapwood-heartwood boundary and is reduced from this boundary to the pith and cambial zone. The content of flavonol, however, is only about 4% of the flavanonol content. It is also remarkable that flavonol measured in alkali medium shows higher values compared to the content in neutral medium. This result is the converse of flavanonol measured in the same media. It is well known that some phenolic compounds are changed to coloring matter in alkaline solution and light irradiated may be to absorbed at longer wave lengths region. Probably, the absorbance of the stock solution is strongly affectly by compounds other than the known flavonols in alkaline medium and the measured absorbance gives a higher value than that of neutral medium. In conclusion, the method in neutral medium is more advantageous to determination of flavonols compared with measurement in alkaline medium.

According to these experimental results, the pattern of flavonol distribution in the disk is very similar to the variation of flavanonol but the flavanonol content is considerably higher than the flavonol content. The flavononol content is calculated to be over twenty times higher than the flavonol content. The distribution pattern of phenolic compounds in wood is evidently in agreement with that in Douglas-fir, western larch and willow[13].

It has already been mentioned by KONDO[11] that a rot of the heartwood of larch in the field has arisen in the ratio of twenty to one. This phenomenon also seems to be in agreement with the effect of higher flavonoid content in the heartwood.

Within the limits of this work, it cannot definitely be said why these flavonoids gradually increase from the cambial zone to the sapwood-heartwood boundary and then why these contents are at a maximum on the heartwood side of this boundary, and further, why these compounds slowly decrease toward the pith. A few aspects of the proliferation of the so-called heartwood constituents in heartwood have been reported[14],[15],[16] and such research has attracted the attention of many researchers.

If taxifolin and aromadendrin are the end products in the biosynthetic pathway, this does not easily explain why the content of flavanonols is reduced toward the pith. In our first experiment of the series, we have observed that flavanonols are converted to water insoluble red colored material when they were treated with an aqueous methyl alcohol which was used without purification. This substance was similar to the red colored material from inner bark. The formation of this compound seems to be effected by temperature, pH, and the presence of water but it is necessary to elucidate these relationships more carefully in a future study.

If the red colored compound, which may be related to the pigmentation of heartwood, is created in the heartwood and the content of flavonoids is decreased in the same situation, it is possible that some of these flavonols served in heartwood are converted into some other colored material, such as a catechin polymer or flavanonol—other compound co-polymers in the vegetable tannins.

Conclusion

From the facts described above, the distribution pattern of the flavonoids in the larch disk gradually increases from S_2 to S_1 in the sapwood and increases considerably from S_1 to H_3 at the sapwood-heartwood boundary. The content of flavonoids is highest at this boundary and then slowly decreases from H_3 to pith in the heartwood. The flavanonol content is about 1% in the sapwood and about 6% in the heartwood (H_3). This pattern of flavanonol content agrees well with that of Douglas-fir and western larch, since the content of taxifolin in the heartwood was 1.6% in Douglas-fir and 1.8% in western larch.

When the content of flavanonol in the bark was measured in neutral medium, a large absorbance was measured because of absorbance by some compounds other than the known flavanonols. Considering the fungicidal activity of larch heartwood and the inhibition cf calcium-base sulpite pulping, the remarkable content of flavonoids in the heartwood should not be overlooked. Also, the relationship between the proliferation of heartwood constituents and the formation of heartwood may be a problem one should investigate. Since these sconstituents gradually decrease from the sapwood-heartwood boundary to pith in heartwood, as mentioned bofore, it is assumed that these compounds are converted to other substances in some way. Furthermore, the chemical studies of the heartwood constituents have important effects onthe utilization of larch. It will be useful in solving the mechanism ofheartwood formation to clarify the increase of these materials at the sapwood heartwood boundary.

In the future, more detailed experimental research is required.

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摘要

前報に引続き，カラマツ *Larix leptoliepis* のフラボノイドの含有量を吸光光度法を用いて定量した。さらに，樹幹内の半径方向に如何なる分布状態をもつかを，併せて検索した。

1. 既に同定された4種の化合物タキシホリン，アロマデンドリン，ケルセチンおよびケンフェロールの検量線の作成を試みたが，各化合物の紫外線吸収の極大値はタキシホリンおよびアロマデンドリン，ケルセチンおよびケンフェロール各々同じ波長領域に存在するため，4種の検量線を作成することは出来なかった。したがって，試料中のフラバノノールの定量のためにタキシホリンの検量線を用い，一方，フラボノールの定量にはケルセチンの検量線を用いた。得られた検量線（第6図および第7図）から最小自乗法により回帰方程式を作成した。この方程式は次の通りである。

○タキシホリン

中　　性 (294 mμ):　$Y = 1.4165\,x - 0.01037$

アルカリ性 (326 mμ):　$Y = 1.9958\,x - 0.01255$

○ケルセチン

中　　性 (360 mμ):　$Y = 2.201\,x - 0.02016$

アルカリ性 (392 mμ):　$Y = 1.1196\,x - 0.0048$

Y:　吸光度 $(-\log T)$

x:　稀釈溶液中の濃度 (10^{-4} mol)

2. 胸高で採取した円板の各部位に含有されるフラボノイドは中性法，アルカリ法で各々測定された（第3表および第4表)。フラバノノールについて，中性法での測定値はアルカリ法で測定した値より高い値を示した。とくに辺・心材境界の心材側 (H_3) においては中性法，アルカリ法共に最高の値を示し，各々6.87%，5.82% であった。この値はタキシホリンとしてはかなりの量を示すが，抽出液中に存在する同じフラバノノールのアロマデンドリ，フラボノール類又は他の少量の化合物の影響と思われる。しかし，アルカリ溶液中でのフェノール類の挙動から考えると326 mμ での測定においては，フラボノールや他の化合物の影響がより少なく，フラバノノールの定量に際してはアルカリ法での測定が中性法における測定より優位と考えられる。一方，フラボノールの含有量は H_3 において，中性法で0.215%，アルカリ法で0.260% の値を示した。フラバノノールとは逆にアルカリ法での値が高くなっている。アルカリ中でのフェノール類は一般に可視部側で吸収するため，この作用の結果，アルカリ法の測定値が高い値を示したものと考えられる。したがって，フラボノールの定量には中性法の方がより適していると考えられる。フラボノールはフラバノノールに比して僅かに4% であった。

3. フラボノイドの半径方向における分布は形成層付近より心材に向って徐々にその含有

量が増加し，辺・心材境界で急激に増加し，その境界の心材側（H_3）で最高に達し，それから以後髄に向って徐々に減少して行く型を示した（第8図および第9図)。この分布型はアメリカ西部カラマツやダグラスファーのタキシホリンの分布型とよく一致している。

4. 樹皮（B_i）のフラバノノールは中性法での測定により7.06%の値を示すが，アルカリ法で3.01%と極端に低い結果を示した。このことから内皮に含有される化合物はむろしフラバノノール型の化合物が主成分でない事を示している。外皮についても同様の結果を得た。

以上の結果，心材成分といわれているこれらの化合物が辺・心材境界で急増する現象から考えて，心材形成に何らかの関係をもつと推察される。