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Citation	木材研究 : 京都大学木材研究所報告 (1969), 48: 15-30
Issue Date	1969-12
URL	http://hdl.handle.net/2433/53045
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Type	Departmental Bulletin Paper
Textversion	publisher

Biochemical Aspects of Heartwood Formation With Special Reference to the Site of Biogenesis of Heartwood Compounds*

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樋口隆昌**・恩田洋志***・藤本幸夫****：心材形成の生化学的的局面，
特に心材物質の生成部位

Introduction

Polyphenols, flavonoids and terpenoids are known as typical heartwood compounds, and the heartwood has been recognized as one of main sources from which new compounds are being isolated by plant chemists. In particular, the heartwood of tropical woods is known to contain a variety of compounds whose structural formulae and physiological activities remain obscure.

Common compounds in the heartwood of trees in temperate zone, however, are polyphenols, flavonoids and terpenoids, such as hydrolyzable- and condensed tannins and resin acids as well as some species-specific compounds. Therefore, elucidation of mechanism and site of biogenesis, and of physiological characteristics of these common compounds in wood should offer us a clue to solve the problem of heartwood formation.

In previous papers¹⁻³⁾ the pattern of carbohydrate metabolism and of activity changes of some enzymes related to polyphenol formation in wood tissues during differentiation and transformation of cambial tissue to heartwood were examined, and a possible activation of some enzymes related to the aging process such as hydrolases and oxidases was discussed.

In the present paper, qualitative and quantitative variations in amounts of lipids and terpenoids accompanied by heartwood formation, the incorporation of radioactive acetate, mevalonate, glucose and phenylalanine into wood tissues, and a possible translocation of precursors to intermediate wood were examined. The results are discussed in connection with heartwood formation.

* Studies on the mechanism of heartwood formation. VIII.

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Materials and Methods

Conifers (*Cryptomeria japonica* D. DON, *Chamaecyparis obtusa* ENDL), and broad-leaved trees (*Fagus crenata* BLUME, *Quercus mongolica* FISCH. var. *grosseserrata* REHD. et WILS., *Magnolia obovata* THUNB.) were felled in the experiment forest of Gifu University in May, 1967 and in May and September, 1968. The cut surfaces of the tree trunks were sealed with paraffin immediately to protect against desiccation and oxidative degradation of living cells of woody tissues, transported to the laboratory and kept in a cool room.

The wood discs (3 cm thick) were cut out from the respective tree trunks, and the sticks (width, 2 cm) through center of the wood discs were cut, and the cambial zone, sap-, intermediate- and heartwood of the sticks were separated immediately.

Measurement of respiration

Sections (0.1 mm thick) of the cambial zone, sap-, intermediate- and heartwood were made in the tangential plane using a sliding microtome. The sections (500 mg) thus obtained were suspended in 2 ml of a phosphate buffer and the O₂-uptake by the sections in following systems were measured at 30°C for 2 hours in 15 minutes intervals.

System 1	Main compartment	Side room	Center well
	Wood sections (500 mg) in 2 ml of 0.1 M phosphate buffer (pH 6.8)	Dist. water (0.2 ml)	KOH (15%) sol. (0.2 ml)
System 2	Same in the above	3N H ₂ SO ₄ (0.2 ml)	Dist. water (0.2 ml)

After completion of the O₂-uptake measurement sulfuric acid in the side room of Warburg's flask in the system 2 was tilted into the center well, the volume of carbon dioxide liberated was measured and the R.Q. (respiratory quotient) of the respective samples was calculated.

Measurement of radioactivity of carbon dioxide formed in respiratory breakdown of acetate-¹⁴C

Wood sections prepared as mentioned above were incubated in a phosphate buffer (pH 5.0) containing 0.1 μ C of acetate-1-¹⁴C and -2-¹⁴C at 30°C for 3 hours, respectively. Then KOH solution containing radioactive carbon dioxide in the center well was transferred quantitatively into the test tube in which 20 mg of K₂CO₃ was previously dissolved. The K₂CO₃ was converted to BaCO₃ by adding an excess amount of BaCl₂ solution and the BaCO₃ precipitated was filtered by a glass filter with a filter paper. A portion of the BaCO₃ formed was taken, and its radioactivity was measured by a gas-flow counter (Aloka Model TDC-1). The total activity of the carbonate from the respective acetates and C₂/C₁ ratio were calculated.

Administration of acetate-2-¹⁴C, mevalonate-2-¹⁴C glucose-G-¹⁴C and phenylalanine-G-¹⁴C into wood tissues

1) Wood sections (5 g) prepared from respective parts were incubated for 5 hours at 30°C in 5 ml of the phosphate buffer (pH 6.8) containing each 2 μ C of acetate-2-¹⁴C, mevalonate-2-¹⁴C, glucose-G-¹⁴C and phenylalanine-G-¹⁴C, respectively and the tissues were washed with a running water throughly and air-dried.

2) As illustrated in Fig. 1, small holes (3.0 mm \times 2.0 cm) were made at the outermost sap-, inner- and inner-most sap- and intermediate wood of the wood discs (5 cm thick) by using an electric drill. The holes were filled with a defatted cotton previously sterilized, and acetate-2-¹⁴C, mevalonate-2-¹⁴C, glucose-G-¹⁴C and phenylalanine-G-¹⁴C (each 3.0 μ C) were injected into the holes.

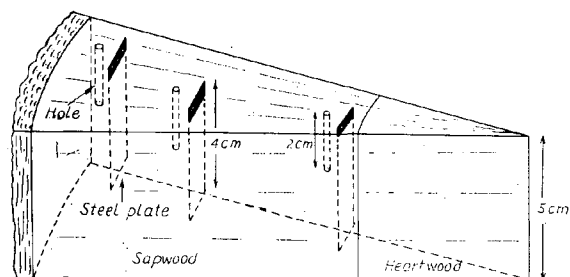


Fig. 1. Administration of radioactive compounds into wood blocks.

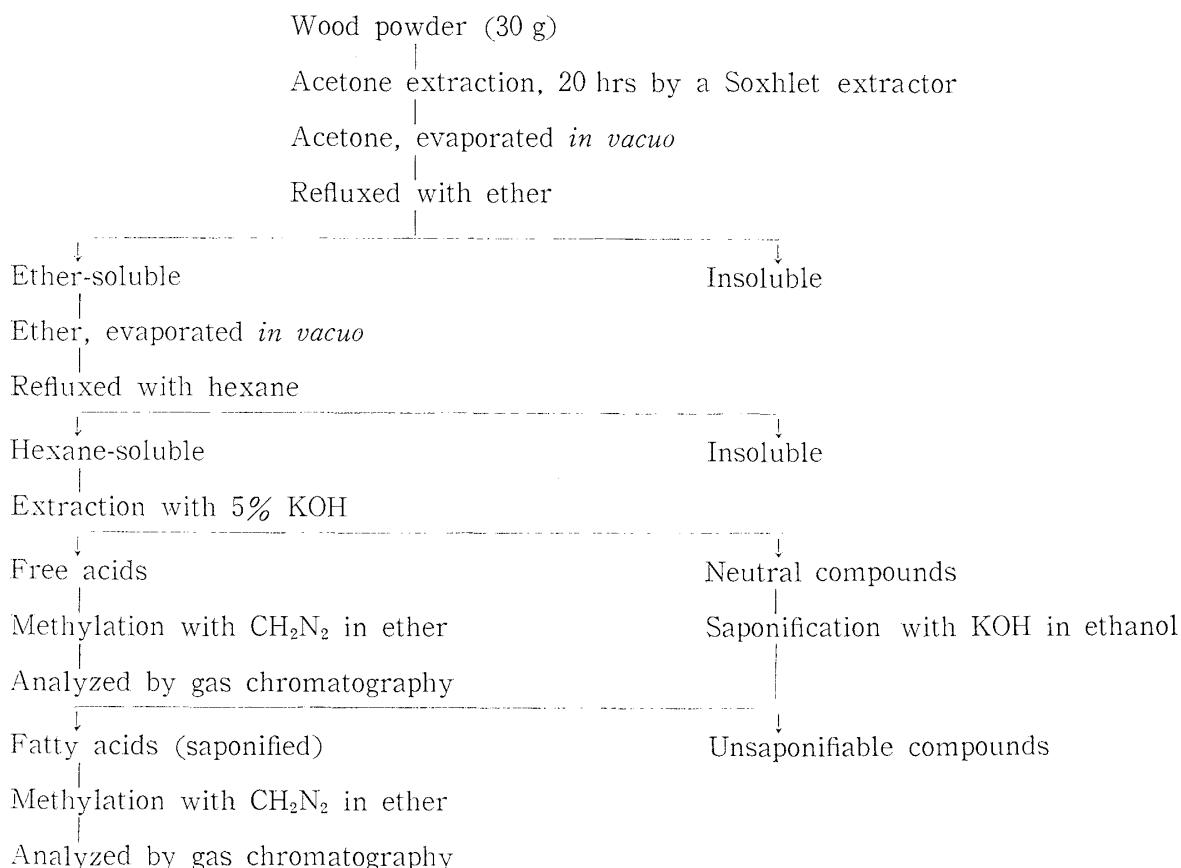
A piece of steel with a sharp edge (4 cm \times 4 cm) was driven into the wood discs 2 cm inside the holes to interrupt the translocation of the compounds to centripetal direction by ray cells. The wood discs were kept at room temperature for 14 days and then the wood adjacent the hole and an inner part of the steel barricade were cut out, separately and pulverized.

Fractionation of wood extractives

The tissues and wood powder prepared from the wood discs administered with the radioactive compounds described above were refluxed with acetone for 3 hours, the acetone of the extracts was evaporated and the residue was dried over P₂O₅ in a desiccator. The extracts were separated into the hexane-, ether- and acetone-soluble fractions, successively, and the radioactivity of each fraction was measured. Moreover, a portion of each fraction was analyzed by paper and thin layer chromatography.

Quantitative determination of acetone extractives of wood

Wood powder (30 g) prepared from the cambial zone, sap-, intermediate- and heartwood, respectively was extracted with acetone and the extractives were separated into free fatty acids, esterified fatty acids and unsaponifiable compounds, and then these were analyzed as follows:



Gas chromatography of fatty acids

Free fatty acids and fatty acids liberated by saponification of esterified acids were methylated with diazomethane in ether.⁴⁾ The methyl esters of the acids were analyzed using a gas chromatograph device (Yanagimoto 550T) with F.I.D. detector. A stainless steel column (3 mm ϕ × 1.5 m) containing a chromosorb W coated with silicon gum (SE 52) was used and the chromatograph was run from 160°C to 280°C provided with 4°C/min. temperature rise, and 35 ml/min. flow rate of carrier gas (nitrogen).

Paper and thin layer chromatography of the extractives

The ether- and acetone-soluble fractions were analyzed by paper and thin layer chromatography using a mixture of toluene, ethylformate and formic acid (5:4:1, v/v) and a mixture of xylene, methyl ethyl ketone and formamide (25:25:1, v/v) as main solvents. For the detection of phenolic compounds on the chromatogram ferric chloride-ferricyanide and diazotized *p*-nitroanilin were usually sprayed and a UV-lamp was also used.

For the analysis of hexane-soluble fraction (mainly free and esterified fatty acids and resin acids) a silicic acid paper was used and the paper was irrigated with a mixture of hexane and ether (19:1, v/v), and the spots were detected with iodine

vapor. The fraction was also analyzed by thin layer chromatography using isopropyl ether as a solvent and the compounds were detected by spraying phosphomolybdic acid in methanol and by heating the chromatograms at 120°C for 5 minutes.⁵⁾

Radioautography of extractives

Paper chromatograms of the hexane-, ether- and acetone-soluble fractions obtained from the extracts with radioactive compounds were submitted to a radioautographic scanner (Aloka Model TRM-1).

Results and Discussion

Sites of biogenesis of heartwood compounds

Many investigations have been carried out in order to elucidate the sites in which heartwood compounds are synthesized. The experiments^{6,7)} with radioactive sucrose applied into cambial tissues of living tree trunks have shown that heartwood compounds can be synthesized in wood not in other tissues such as leaves, and tissue culture experiments⁸⁾ supported these results, which subsequently indicate the biosynthetic ability of woody tissues for the heartwood compounds.

In the tracer experiments described above, however, radioactive sucrose and the compounds synthesized from the sucrose at a certain part of tissues can be translocated to both centripetal and centrifugal directions through ray cells, and the actual sites of the synthesis can not be determined by these experiments. Concerning with these problems the experiment with tissue culture also can not give a satisfactory answer but gives only information of biosynthetic ability for heartwood compounds.

In the present experiment, two methods have been undertaken to eliminate the effects of translocation of precursors and to elucidate the actual sites of synthesis of heartwood compounds.

Experiment by wood sections

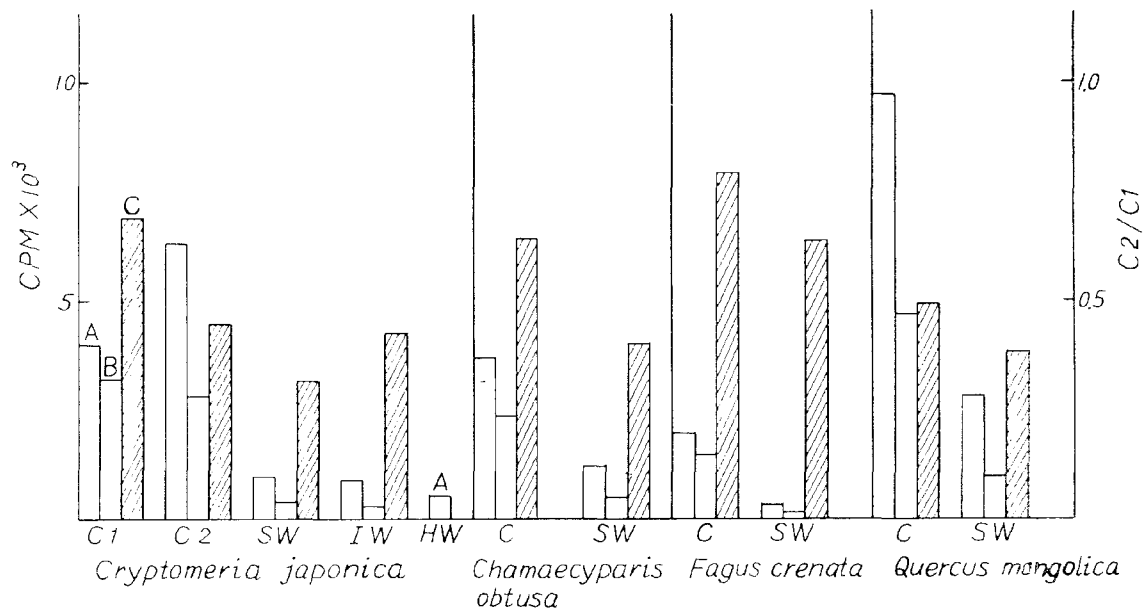
In the first experiment metabolic and synthetic activities of wood sections prepared separately from the cambial zone, outersap-, innersap-, intermediate- and heartwood were examined.

As reported previously the high respiratory O₂-uptake by the cambial tissues decreased to a very low level in the sap- and intermediate wood and practically no appreciable O₂-uptake was found in the heartwood. As given in Table 1, the R.Qs. of the cambial tissues were around 0.5-0.7 and the results indicated a possible contribution of fatty acids as a metabolic substrate as well as sugars for respiration in the cambial tissues. The R.Qs. of sap- and intermediate wood, however, could not be calculated because both the volumes of O₂-uptake and CO₂-output by these tissues were quite low and then reproducible values were scarcely obtained.

Table 1. Respiration of cambial tissues.

	Date	O ₂ -uptake (μ l/500 mg/hr)	R.Q. value
<i>Cryptomeria japonica</i>	May 20	98	0.61
	21	105	0.64
	24	103	0.58
	June 1	91	0.68
<i>Chamaecyparis obtusa</i>	May 22	94	0.54
	25	126	0.58
<i>Fagus crenata</i>	June 1	78	0.51
<i>Quercus mongolica</i>	May 26	98	0.56
	28	104	0.53

The total radioactivity of carbon dioxide formed in the metabolic degradation of acetate-1-¹⁴C and -2-¹⁴C by the sections of respective woody tissues and C₂/C₁ ratio are shown in Fig. 2. The pattern of changes in metabolic activity was quite similar to that of respiratory O₂-uptake. However, the ratios in sapwood were generally lower than those in cambial tissues through conifers and broad-leaved trees indicating a possible participation of glyoxylic acid cycle⁹⁾ in sapwood, which is concerned with the conversion of fatty acids to sugars, as well as TCA cycle. Thus the results further support that fatty acids, frequently occurring in ray parenchyma of sapwood as gly-

Fig. 2. Metabolic degradation of acetate-¹⁴C by wood sections.

A, CO₂ from acetate-1-¹⁴C, B, CO₂ from acetate-2-¹⁴C, C, C₂/C₁ ratio, C₁, outer parts of cambial zone, C₂, inner parts of cambial zone, SW, sapwood, IW, intermediate wood, HW, heartwood.

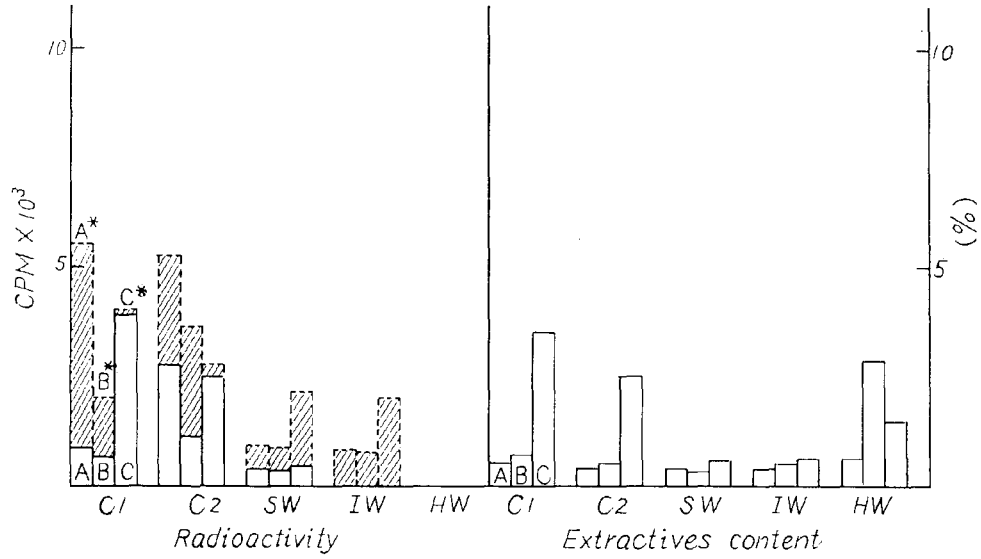


Fig. 3. Incorporation of acetate-2-¹⁴C into various fractions of wood extractives of *Cryptomeria japonica*
 A, hexane-soluble fraction (3 hrs experiment using 0.1 μ C of acetate),
 B, ether-soluble fraction, C, acetone-soluble fraction.
 A*, hexane-soluble fraction (5 hrs experiment using 2 μ C of acetate),
 B*, ether-soluble fraction, C*, acetone-soluble fraction.

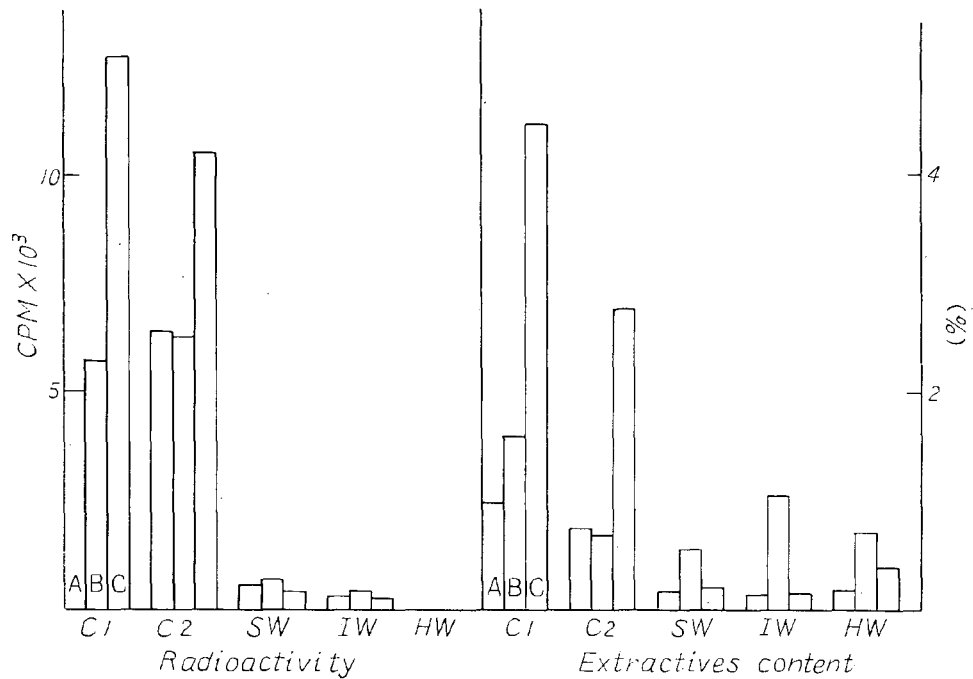


Fig. 4. Incorporation of acetate-2-¹⁴C into various fractions of wood extractives of *Quercus mongolica* (3 hrs experiment using 0.1 μ C of acetate).
 Marks denote the same as in Fig. 3.

cerides, are consumed as substrates for respiration and as a carbon source in the biosynthesis of secondary products.

Anyway, the results indicated that the metabolic activities of the sap- and intermediate wood were quite low, except for the high metabolic activity in the cambial tissue.

Acetic acid, mevalonic acid and phenylalanine are known to be obligatory precursors of fatty acids, terpenoids, polyphenols and flavonoids, respectively. Then the incorporation of these compounds into the heartwood compounds by the woody tissue was examined.

As shown in Figs. 3 and 4, the incorporation of acetate- ^{14}C into the hexane-soluble (mainly lipids and terpenoids), ether-soluble (phenolics) and acetone-soluble (catechins, leucoanthocyanins and hydrolyzable tannins etc.) fractions were quite high in the cambial tissues of the both conifers and broad-leaved trees in agreement with the experimental results of respiration. However, the incorporations in other tissues were also reasonably high, especially in the experiment of longer reaction time (5 hrs) with a larger amount ($2\ \mu\text{C}$) of radioactive compounds (Fig. 3). The results indicate considerable ability for sapwood to synthesize heartwood compounds but no increased incorporation was observed in the intermediate wood.

In contrast to these results, the amounts of extractives in heartwood were much higher than those in sap- and intermediate wood as shown in Fig. 5, and moreover no incorporation of these compounds into the extractives of the heartwood was observed. This may be explained as such a way that in living trees precursors such as malonyl CoA (acetyl CoA), dimethylallyl pyrophosphate and cinnamic acid synthesized in sapwood as well as in cambial tissues are incorporated into lipids, terpenoids and flavonoids in whole woody tissues, although the incorporation ability for the cambial tissues is greater than that for the other tissues. Occurrence of the high amounts of extractives in heartwood should be interpreted in terms of an accumulation of translocated compounds through ray cells but not in terms of a high synthetic activity around the intermediate wood.

Experiment with wood discs

In the second experiment, to elucidate the sites of biogenesis of heartwood compounds, conversion of acetate- $2\text{-}^{14}\text{C}$, mevalonate- $2\text{-}^{14}\text{C}$, glucose- $\text{G-}^{14}\text{C}$ and phenylalanine- $\text{G-}^{14}\text{C}$ to lipids, terpenoids and phenolics in the respective wood tissues were examined by use of the wood discs previously injected with these radioactive compounds as described in experimental parts.

As shown in Figs. 5, 6 and 7 through conifers and broad-leaved trees the incorporation of these compounds into the various fractions of the acetone extracts were similarly quite high indicating the sufficient biosynthetic ability of the respective tissue

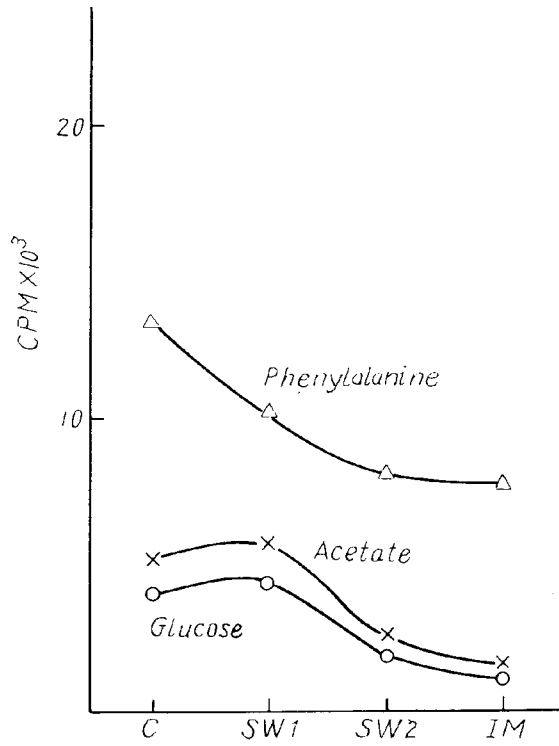


Fig. 5.

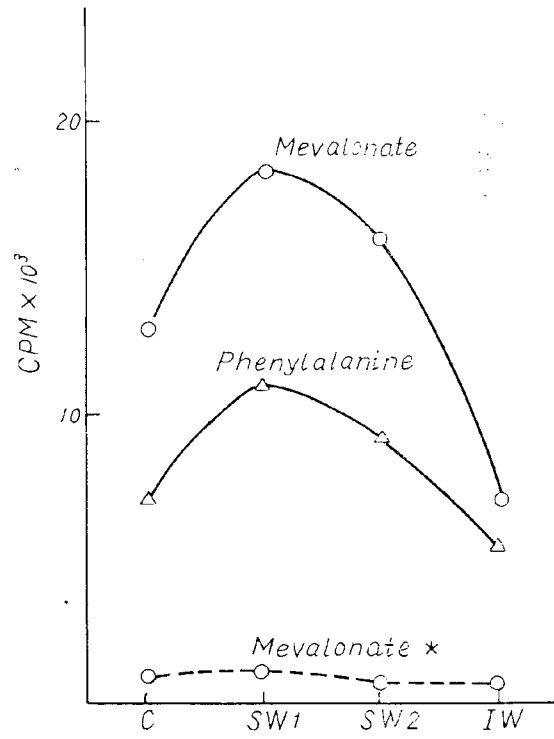


Fig. 6.

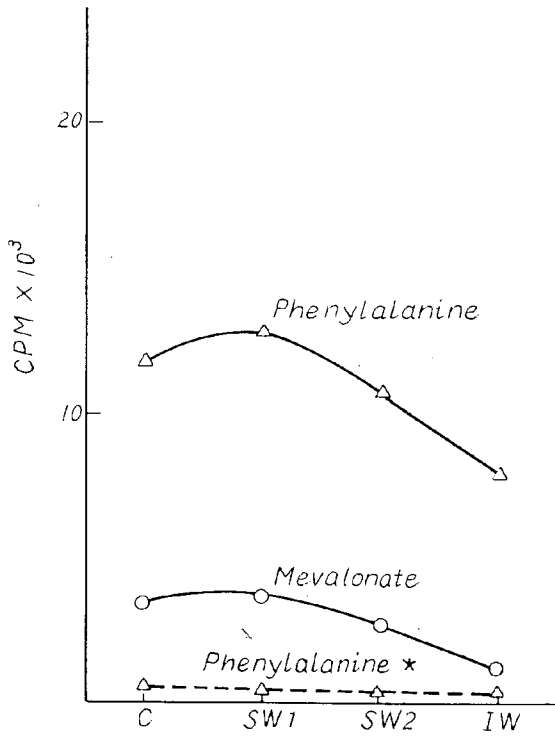


Fig. 7.

Fig. 5. Incorporation of radioactive compounds into wood extracts of *Magnolia obovata*.

C, cambial zone, SW1, outer sapwood, SW2, inner sapwood, IW, intermediate wood.

Fig. 6. Incorporation of radioactive compounds into wood extracts of *Cryptomeria japonica*.

* wood within steel plate.

Fig. 7. Incorporation of radioactive compounds into wood extracts of *Quercus mongolica*.

* wood within steel plate.

for the extractives, and the radioactivity of the extractives of the tissues beyond the steel inserted was quite low. However, the radioactivity of the extractives of control without insertion of the steel barricade was also quite low at the corresponding parts of the wood, and this suggests rather poor translocation of precursors through ray cells under the present experimental conditions.

Tables 2 and 3 show that mevalonic acid and phenylalanine were incorporated mainly into the hexane-soluble, and both ether-soluble and acetone-soluble fractions, respectively, whereas acetate and glucose were incorporated into every fraction rather consistently. These facts are in good agreement with the findings on the role of these compounds as precursors of terpenoids and phenolic compounds.

Thus, the results seem to explain that the precursors of the heartwood compounds are synthesized in the tissues through sap- to intermediate wood as well as in the cambial tissues. Although the occurrence of shikimic acid, cinnamic acids and catechins in cambial sap and the transportation of phenolic compounds through ray cells have been established, the contribution of the translocation of phenolics and terpenoids

Table 2. Incorporation of radioactive compounds into various fractions of wood extractives of *Magnolia obovata*.

	Radio activity (%)		
	Hexane-sol.	Ether-sol.	Acetone-sol.
Acetate-2- ¹⁴ C			
Cambial zone	50.5	13.6	35.9
Outer sapwood	46.4	31.3	22.3
Inner sapwood	23.7	22.0	54.3
Intermediate wood	22.4	20.9	26.7
Glucose-G- ¹⁴ C			
Cambial zone	38.1	25.2	36.7
Outer sapwood	20.4	24.6	55.0
Inner sapwood	16.1	27.8	56.1
Intermediate wood	23.0	26.6	50.4
Mevalonate-2- ¹⁴ C			
Cambial zone	70.9	26.8	2.3
Outer sapwood	65.8	28.6	5.6
Inner sapwood	57.6	33.2	9.2
Intermediate wood	60.8	34.2	5.0
Phenylalanine-G- ¹⁴ C			
Cambial zone	0	37.3	62.7
Outer sapwood	2.3	55.2	42.5
Inner sapwood	3.8	57.2	39.0
Intermediate wood	3.8	65.2	31.0

Table 3. Incorporation of radioactive compounds into various fractions of wood extractives of *Cryptomeria japonica*.

	Radio activity (%)		
	Hexane-sol.	Ether-sol.	Acetone-sol.
Acetate-2- ¹⁴ C			
Cambial zone	44.5	22.5	33.0
Outer sapwood	27.0	24.5	48.5
Inner sapwood	27.3	25.2	47.5
Intermediate wood	26.5	24.8	48.7
Glucose-G- ¹⁴ C			
Cambial zone	18.5	28.2	53.3
Outer sapwood	22.1	28.3	49.6
Inner sapwood	27.2	25.6	47.2
Intermediate wood	20.6	30.5	48.9
Mevalonate-2- ¹⁴ C			
Cambial zone	60.0	20.8	19.2
Outer sapwood	65.0	28.0	7.0
Inner sapwood	70.6	25.3	14.2
Intermediate wood	48.4	32.8	18.8
Phenylalanine-G- ¹⁴ C			
Cambial zone	4.4	43.7	51.9
Outer sapwood	2.2	32.5	65.3
Inner sapwood	3.9	41.2	54.9
Intermediate wood	2.1	67.6	30.3

on the heartwood formation is rather limited, and the compounds are mostly synthesized *in situ* from sugars.

At the first stage of biosynthesis of flavonoids, the condensation of 3 malonyl CoA with cinnamoyl CoA to form chalcones has been presumed. And participation of isopentenylpyrophosphate and dimethylallyl pyrophosphate in the biosynthesis of terpenoids was demonstrated. Thus, many exergonic reactions which should couple with ATP producing systems are involved in the biosynthesis of heartwood compounds. Even in shikimate and mevalonate pathways participating in the formation of the precursors of flavonoids and terpenoids several molecules of ATP are required. Therefore, it will be generally concluded that a sufficient amount of chemical energy is required for the synthesis of the precursors for heartwood compounds.

Although the number of living cells is quite small, in the sap- and intermediate wood; and apparent metabolic activity for both is considerably lower than that for cambial tissue, the metabolic activity per living cell must be high enough to synthesize the precursors and heartwood compounds.

As it has been known that resin acids are synthesized in epicelium cells and that the epicelium cells live in sapwood, the acid should be synthesized in the cells. Fig. 6 demonstrates the case clearly.

Mevalonate was incorporated considerably into various compounds of the hexane-soluble fraction of both sapwood and cambial tissues of *Cryptomeria japonica*. As shown in Fig. 8, the hexane-soluble fraction gave several radioactive spots on the radioautogram. Each spot was eluted, and several identification procedures including UV analysis have suggested that these compounds are terpenoids. Xantoperol, ferruginol, sugiol, cryptopimaric acid and isodextropimaric acid were used for comparison as authentic compounds. One of the radioactive spots (A) the most active compound in ether-soluble fraction showed quite similar Rf values and UV spectrum to those of sugiol.¹⁰⁾ How-

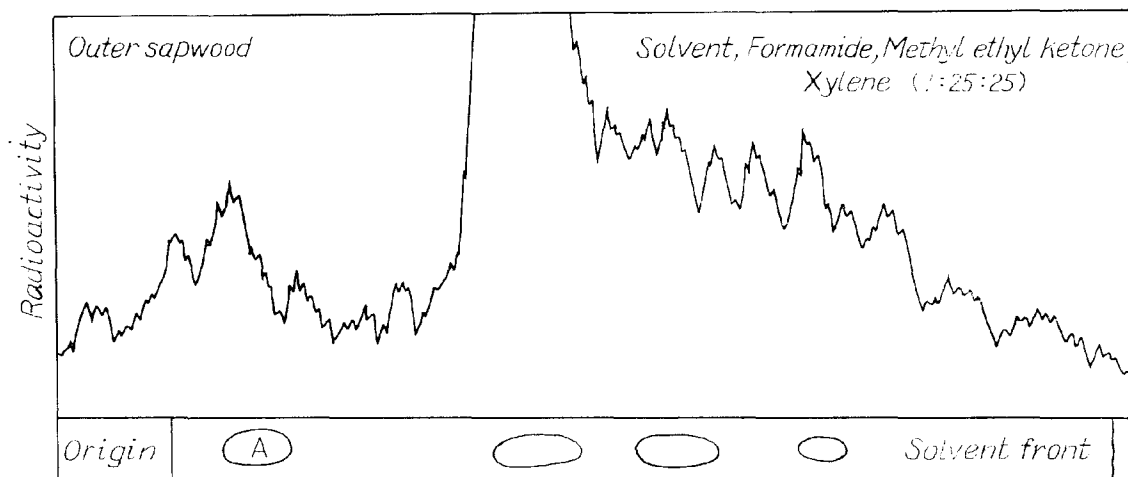


Fig. 8. Radioautogram of hexane-soluble fraction of wood extractives of *Cryptomeria japonica* administered with mevalonate-2-¹⁴C.

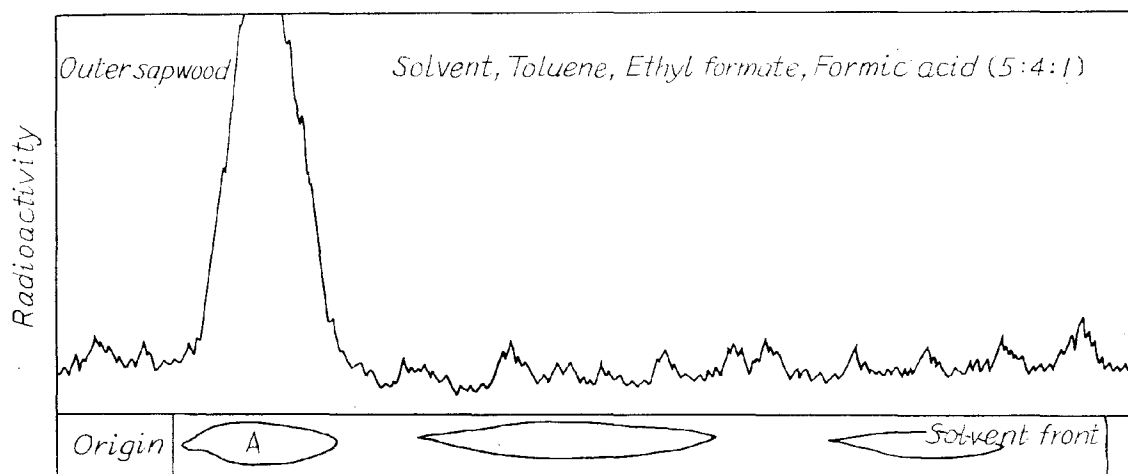


Fig. 9. Radioautogram of acetone-soluble fraction of wood extractives of *Quercus mongolica* administered with phenylalanine-G-¹⁴C.

ever, further identification could not be performed because of small amount of the compound.

A radioautogram of the acetone-soluble fraction of sapwood of *Quercus mongolica* which previously administered with phenylalanine was shown in Fig. 9. The spot A gave typical phenol reactions, and chromatographic behavior and UV analysis suggested it to be a hydrolyzable tannin,¹¹⁾ but not gallic and ellagic acids.

Thus, it may be reasonable to understand that sugars, in particular sucrose, from leaves are translocated to the cambial tissues and sapwood through ray cells and the heartwood compounds are synthesized *in situ*, and during the transformation of sapwood to intermediate wood and to heartwood, hydrolysis of glycosides and oxidative polymerization of catechins, hydrolyzable tannins and leucoanthocyanins should occur in the ray parenchyma of the intermediate wood. The activations of hydrolases and oxidases in the intermediate wood must be participated to such final reactions.

Gas chromatography of fatty acids in the extractives

Fig. 10 shows the charts of gas chromatography of the methyl esters of fatty acids

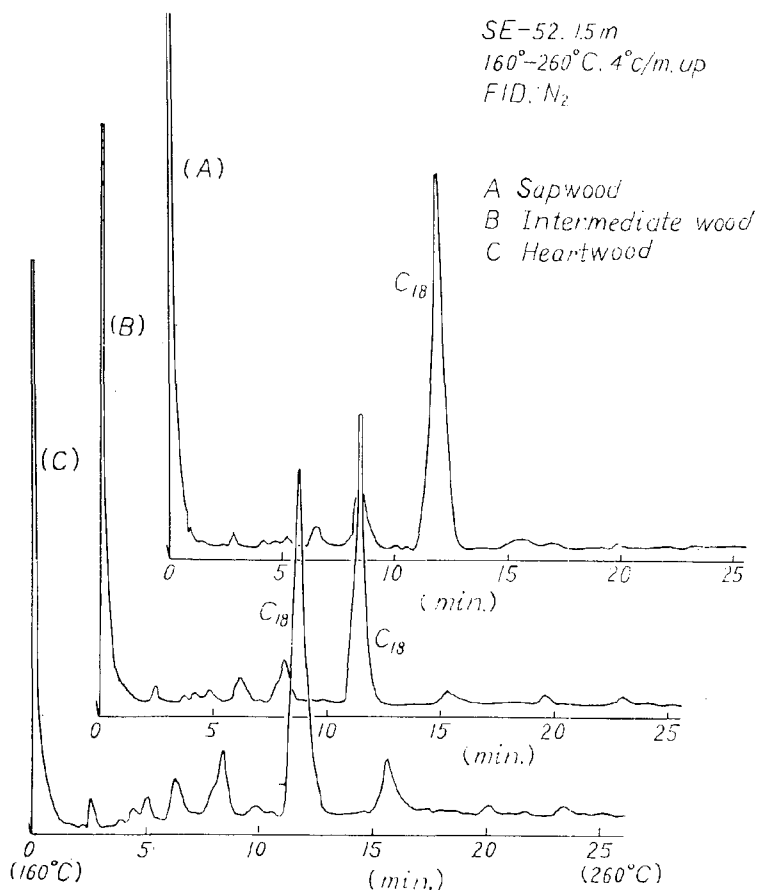


Fig. 10. Gas chromatograms of the esterified fatty acids of *Quercus* sp. as methyl esters.

obtained by the saponification of esterified acids from the wood of *Quercus mongolica*.

As shown in this figure C₁₂-C₂₄ acids were found to be common to the broad-leaved trees. The figure also shows that the qualitative and quantitative compositions of fatty acids of the trees are very similar through sapwood to heartwood, although the amounts of free acids in the intermediate- and heartwood increased complementarily against the decrease of esterified acids in those tissues (Tables 4 and 5). No evidence for resin acids was found.

Table 4. Composition of petroleum ether-soluble fraction of wood.

	<i>Fagus crenata</i>			<i>Quercus mongolica</i>		
	SW	IW	HW	SW	IW	HW
Petroleum ether-sol.*	0.16	0.14	0.20	0.20	0.17	0.22
Neutral compounds**	87.6	83.1	81.7	73.5	70.9	61.6
Free acids**	12.4	16.9	18.3	26.5	29.1	38.4
Esterified acids**	57.6	54.5	45.0	46.9	41.7	24.3
Unsaponifiable**	32.1	33.8	36.7	22.6	28.2	28.3

* per cent of dry wood, ** per cent of petroleum ether-soluble fraction. SW, sapwood, IW, intermediate wood, HW, heartwood.

Table 5. Composition of petroleum ether-soluble fraction of wood.

	<i>Cryptomeria japonica</i>			<i>Chamaecyparis obtusa</i>		
	SW	IW	HW	SW	IW	HW
Petroleum ether-sol.*	0.19	0.37	0.90	0.37	1.00	4.68
Neutral compounds**	73.3	70.1	54.0	87.9	66.7	52.8
Free acids**	26.7	29.9	36.0	18.1	33.3	39.2
Esterified acids**	39.7	30.0	19.9	57.1	25.6	13.8
Unsaponifiable**	33.6	33.6	30.1	30.7	41.1	39.0

* per cent of dry wood, ** per cent of petroleum ether-soluble fraction. SW, sapwood, IW, intermediate wood, HW, heartwood.

Among the fatty acids of both free and esterified forms the amounts of C₁₈ acids corresponding to oleic, linoleic and linolenic acids were predominant. The increase of free fatty acids toward the intermediate and heartwood in the both trees might be ascribed partly to enzymatic hydrolysis of the esterified fatty acids relating to the metabolism of the acids for the synthesis of secondary compounds and also to acid hydrolysis during aging process of the tissues, particularly in the heartwood without living cells.

Fig. 11 shows the composition of fatty acids from the wood of *Cryptomeria japonica*. The pattern of the composition of fatty acids in sapwood is rather similar to that of broad-leaved trees⁵⁾ but the composition, markedly varied in the intermediate- and

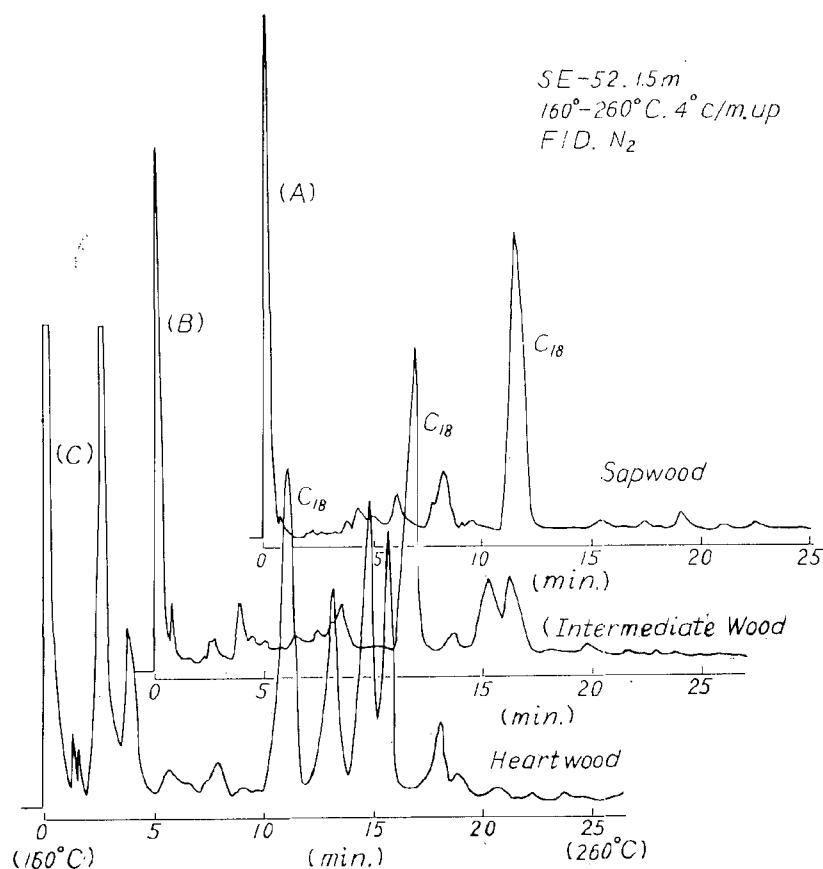


Fig. 11. Gas chromatograms of the esterified fatty acids of *Cryptomeria japonica* as methyl esters.

heartwood. These facts explain that the heartwood compound such as resin acids synthesized in ray cells and some epicelium cells in sapwood were accumulated in the intermediate- and heartwood.

Furthermore, thin layer chromatography of neutral compounds of sapwood from *Cryptomeria japonica*, *Chamaecyparis obtusa*, *Quercus mongolica* and *Fagus crenata* suggests that the main parts of the compounds are composed of triglycerides of fatty acids.

Acknowledgement

The authors wish to thank Prof. Dr. H. IMAMURA and Mr. T. KANO of Gifu University for their helpful advice and discussion on identification of wood extractives and experimental help, respectively. They also thank Prof. Dr. K. HATA of Kagawa University for providing us with xantoperol.

Summary

Two methods of experiment have been carried out to establish the sites of bio-

genesis of heartwood compounds. 1) Wood sections prepared from cambial zone, sap-, intermediate- and heartwood were administered with acetate-2- ^{14}C , mevalonate-2- ^{14}C , glucose-G- ^{14}C and phenylalanine-G- ^{14}C , and incorporation of these compounds into wood extractives was determined. 2) Small holes were made at sap- and intermediate woods of wood discs and radioactive compounds mentioned above were injected into the holes, and the incorporation of these compounds into wood extractives was determined. The incorporation of these compounds into wood extractives of the respective wood tissues were quite high not only at cambial tissues but also at sap- and intermediate woods in both conifers and broad-leaved trees indicating sufficient biosynthetic ability of the respective tissues for the wood extractives. The results suggest that the precursors and heartwood compounds are mostly synthesized in sap- and intermediate woods from sugars. Hydrolysis of glycosides and oxidative polymerization of catechins, hydrolyzable tannins and leucoanthocyanins should occur in ray parenchyma of the intermediate wood during transformation of sapwood to intermediate- and heartwoods. The activated hydrolases and oxidases in the intermediate wood may participate in such final reactions.

摘 要

心材抽出物の木材組織中での生成部位を検討するため、針、広葉樹生材の形成層部、辺材部、移行材部、心材部から得られたマイクローム切片を用い、酢酸-2- ^{14}C 、メバロン酸-2- ^{14}C 、グルコース-G- ^{14}C 、フェニルアラニン-G- ^{14}C のこれら組織への取りこみ量を検討した。なお、これと平行して生材円板の各組織部位に小孔をあけ、この中に上記アイソトープを注入した。切片による実験では各アイソトープは形成層部抽出物中に最も多量に取りこまれたが、辺材部、移行材部の抽出物中にもかなりの取りこみが認められた。円板による実験では辺材部、移行材部抽出物への取りこみが形成層部抽出物への取りこみに匹敵しており、生細胞の少い辺材部、移行材部でも心材抽出物合成に充分な代謝活性を持っていた。したがって心材抽出物は葉で合成された糖が放射組線を通して辺材、移行材に転流され、それらの組織の放射感受細胞によってそれぞれ樹種特有の抽出物に合成され、心材に転流されて蓄積するものと考えられる。

Literature

- 1) HIGUCHI, T., M. SHIMADA and K. WATANABE, J. Japan Wood Research Soc., **13**, 269 (1967).
- 2) HIGUCHI, T., M. SHIMADA and K. WATANABE, J. Japan Wood Research Soc., **13**, 274 (1967).
- 3) HIGUCHI, T., K. FUKAZAWA and M. SHIMADA, Research Bull. College Exp. Forests, Hokkaido Univ., **25**, 167 (1967).
- 4) BROWNING, B. L., Methods of Wood Chemistry, p. 152, Interscience Publisher (1967).
- 5) MINAMI, K. and Y. SABURI, Bull. Tokyo Univ. Forests, **64**, 293 (1968).
- 6) HASEGAWA, M. and T. SHIROYA, The Botanical Magazine, Tokyo **79**, 595 (1966).
- 7) HASEGAWA, M. and T. SHIROYA, IUFRO 14th Congress Munich, (1967).
- 8) HASEGAWA, M., T. HIGUCHI and H. ISHIKAWA, Plant & Cell Physiol., **1** 173 (1960).
- 9) YAMAMOTO, Y., Protein, Nucleic acid, Enzyme, **7**, 9 (1962).
- 10) KONDO, T., H. IMAMURA and M. SUDA, Bull. Agr. Chem. Soc. Japan, **23**, 233 (1959).
- 11) IMAMURA, H., Private communication.