POSSIBLE ORIGINS OF THE METHOXYL CARBON OF LIGNIN FORMED BY *HORDEUM VULGARE*

By

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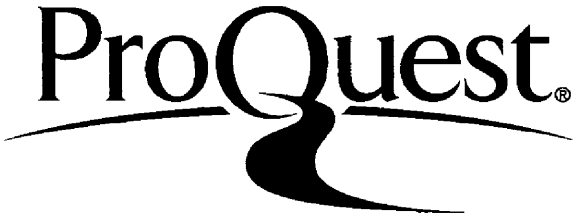

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INTRODUCTION

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In the past several years many investigators have shown the importance of transmethylation reactions in the animal organism (1), but relatively little is known about the role transmethylation plays in plant metabolism. Experiments recently undertaken by Kirkwood and Marion (2), in which they attempted to demonstrate the transfer of methyl groups from choline to the barley plant alkaloid, hordenine, using radioactive tracer techniques, were unsuccessful. However, in recent months it has been shown by Brown (3) that the methyl carbon of methionine may be transferred to the methyl group of nicotine in tobacco (Nicotiana rustica), and it was postulated that a transmethylation reaction was involved. It seemed possible that transmethylation reactions might be involved in the metabolism of other plant products.

The work described here was undertaken in an attempt to study the possible role of transmethylation in the formation of lignin in the plant. Lignin, the substance chosen for study in this investigation, is a constituent of the cell wall of plants. Although the amount of lignin present in a plant increases as the plant grows older, there is an appreciable amount present in comparatively young barley plants.

Lignin is not a definite chemical compound, its composition varying according to the method used in its isolation. Hence a great deal is unknown about its chemical structure and its formation in the plant. Fuchs (4) suggested that pectin, a partially methyl-esterified polygalacturonic acid formed in plant cell walls, acts as the precursor of

lignin, since it had been shown that the percentage of pectins in older, lignified plant tissues was smaller than in young plant tissues. However, as the plant ages, whereas the percentage of pectin decreases, the actual amount of pectin present increases (5). Further, it is known that in lignin the methoxyl groups form an ether linkage, not an ester linkage such as is found in pectin, so a rather profound chemical change would have to take place if lignin were to form from pectin. A more reasonable explanation of its appearance was offered by Hibbert (6). On the basis of the structure of the decomposition products of lignin, he postulated that a fructose derivative can condense with guaiacol in the formation of lignin. Except for a suggestion, made by Klason (7), that formaldehyde can act as a precursor of the methoxyl groups in lignin, little is known about their origin.

Since no satisfactory explanation for the appearance of the methoxyl groups in lignin has been offered, it seemed desirable to learn whether transmethylation was involved in their formation. Although in all previously reported transmethylation studies the compounds investigated contained methyl groups attached to sulfur or nitrogen atoms, it seemed possible that transfer of a methyl group to an oxygen atom could take place, thus forming a methoxyl group.

It was decided in the present study: (a) to feed barley plants appropriately labelled methionine and formate, which are known to act as methyl donors or precursors in the animal organism, (b) to isolate the lignin formed by the plants, and (c), if radioactivity is found, to perform degradations in order to determine the location of the radioactivity in the lignin.

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

Radioactive Precursors

The radioactive tracers used in this work were D,L-methionine containing C^{14} in the methyl group and the sodium salt of C^{14}-formic acid. Methionine was synthesized by reacting D,L-S-benzylhomocysteine with C^{14}- methyl iodide which was obtained from Tracerlab, Inc., under allocation from the United States Atomic Energy Commission. The formate was obtained from Oak Ridge National Laboratory, also under allocation from the United States Atomic Energy Commission.

Preparation of Barley Plants

A variety of Hordeum vulgare known as Bay barley was used in this investigation. According to Phillips and Goss (8) the percentage of methoxyl in barley lignin increased at the fastest rate when the plants were thirty to forty days old. MacDougall and De Long (9) showed that, with respect to lignin content, ninety-four-day-old plants grown in the greenhouse were at about the same stage of maturity as forty-one-day-old plants grown in the field. Consequently the seeds were planted in flats and allowed to grow outside the greenhouse for about thirty days. Plants of this age were from eight to twelve inches tall, variations probably being due to seasonal effects. A commercial plant food mixture was fed as required in a water solution.

Before being fed the radioactive compounds, the plants were given the following treatment. As much of the adhering soil as possible was

carefully removed from the roots by shaking. After soaking in water for about thirty minutes, any dirt left on the roots was washed away under running water. To reduce the number of bacteria present, the roots were soaked in a 0.1 percent solution of Wyandotte detergent germicide No. 1528[1] for about thirty minutes. Following a rinse in distilled water, the roots of each plant were placed in 25 ml. of an inorganic nutrient solution in a 50 ml. Erlenmeyer flask. This nutrient solution was prepared by making a 1:3 dilution of the stock solution, the composition of which is given in Table I. As a further precaution

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTION

Calcium nitrate:	$Ca(NO_3)_2$, 1 g.
Potassium chloride:	KCl, 250 mg.
Magnesium sulfate:	$MgSO_4$, 250 mg.
Ammonium sulfate:	$(NH_4)_2SO_4$, 250 mg.
Ferric chloride:	$FeCl_3$, 2 mg.
Potassium dihydrogen phosphate:	KH_2PO_4, 250 mg.
Distilled water to 1 l.	

against bacterial contamination, three drops of a one percent solution of the detergent germicide were added to each flask. Each flask also contained a suitable amount of the radioactive compound being studied, as will be described later.

In the plants' metabolism of the radioactive compounds there was a possibility of $C^{14}O_2$ being liberated. The plants were grown in a fume

[1] This material was obtained from the Wyandotte Chemical Corp., Wyandotte, Mich., by the Michigan State College Horticulture Department.

hood, in order to prevent the contamination of the air from this source. Two 36-inch, 30-watt fluorescent tubes and a 100-watt bulb, about 15 inches above the plants, were used as a source of light. A light intensity of about 200 foot-candles at the leaves was thus obtained. During the feeding experiments, the light was turned on for about 12 hours out of 24. The volume of the nutrient solution was kept fairly constant by the addition of distilled water.

Isolation of Lignin

Difficulty was encountered in the isolation of lignin, using a modification of the sulfuric acid method of Ost and Wilkening (10). In this method the dried plant tissue was put into 70 percent sulfuric acid for 16 hours at 20° C. or less. The sulfuric acid was then diluted to about three percent, the mixture was hydrolyzed for two hours at 100° C., and filtered, lignin being the precipitate obtained. When this method was used with these young plants, it appeared that complete wetting by the sulfuric acid was not achieved. It was felt that an extraction of the fatty material from the tissue would permit complete contact by the sulfuric acid. However, after an ether extraction was performed on the tissue, the percentage of "lignin" present was still found to be about three times as high as that reported in the literature in work done with comparable plants (8).

MacDougall and De Long (9) found that the absolute methoxyl content of lignin from dried plant tissues was greater than that of lignin from fresh tissues of the same plants. They thought that this was due to the

inclusion of methoxyl-containing carbohydrates in lignin from the dried tissues. The fresh plants used in the present work were pre-treated before placing in contact with the sulfuric acid. This treatment consisted of extractions in a Waring Blendor using various solvents, as suggested by MacDougall and De Long (11). Ether-saturated water was believed by them to be the best non-acid solvent for removal of nitrogen-containing material from young undried tissues. A five percent solution of acetic acid was found effective in removing carbohydrates from young tissue. A constant-boiling (1:2) mixture of ethanol and benzene was used as a solvent for fatty materials present in the tissues. Accordingly, in the present work, a pretreatment of the plant tissues was adopted. The following extractions were performed on the tissues in a Waring Blendor: two 15-minute extractions with ether-saturated water, one 20-minute extraction with five percent acetic acid, two 15-minute extractions with 1:2 ethanol-benzene. A suitable quantity of 70 percent sulfuric acid was added to the dried fiber obtained after carrying out the above procedure and the reaction mixture allowed to stand for about 18 hours at five degrees C. This was done in the cold because it had been shown that carbonization of hydrolyzed carbohydrates takes place at higher temperatures, causing difficulty in the subsequent filtration and producing an impure lignin preparation (12). At the end of 18 hours, the sulfuric acid was diluted to three percent, boiled gently for two hours, keeping the volume constant by adding water, and allowed to cool; the lignin settling out. The lignin was then filtered, using a fritted glass filter, washed

thoroughly, and dried in a vacuum desiccator at room temperature. The lignin content of plants this age was found to be about 4.5 percent on a dry weight basis.

Demethylation of Lignin

To prove that any radioactivity found in the lignin was located in the methoxyl carbon, it was necessary to split off the methyl group and obtain it in the form of a solid derivative suitable for counting. A modification of the method of Phillips (13), in which the methoxyl group, treated with hydriodic acid, yields methyl iodide, was used. In this method the methyl iodide is caused to react with silver nitrate and the precipitate of silver iodide is weighed. However, since in the present work it was necessary to recover the methyl group, the methyl iodide formed was swept into a solution of triethylamine. Previous work done in this laboratory showed that triethylamine reacts quantitatively with methyl iodide to form methyl triethylammonium iodide. Triethylamine is also less volatile than trimethylamine, which was used by Simmonds and co-workers (14).

A modified form of the apparatus described by Pregl (15, 3) was used for demethylation. About 60 mg. of the lignin to be demethylated was weighed into cigarette paper and placed in the reaction vessel with two ml. of phenol, which acted as a solvent for the lignin, and four ml. of 47.3 percent hydriodic acid. Attached to this was a gas-washing bubbler containing 1.5 ml. of the five percent $CdSO_4$-$Na_2S_2O_3$ solution recommended by Pregl to remove the hydriodic acid and iodine. A tube from the

bubbler led to the receiving vessel, which contained a five percent solution of triethylamine in ethanol, cooled in a methyl cellosolve-CO_2 bath to about -75° C.

During demethylation the reaction flask was immersed in a copper oxide bath. A stream of nitrogen was slowly run through the side-arm of the reaction flask and through the demethylation assembly. Experimentation showed that best results were obtained when the temperature of the bath was kept at about 150° C. for 45 minutes, then raised to 200° C. and held there for 30 minutes. The bath was allowed to cool for 15 minutes, during which time a faster stream of nitrogen was passed through the apparatus. At this time the tip of the delivery tube was rinsed with ethanol into the receiving vessel. This vessel was then stoppered and allowed to stand overnight at room temperature. The ethanol solution was taken almost to dryness by heating, the last trace of ethanol and unreacted amine removed in a vacuum desiccator, and a white solid, the methyl triethylammonium iodide, remained. This was weighed and tested for radioactivity.

A methoxyl analysis of lignin from thirty-day-old barley plants was made and the methoxyl content of the lignin was found to be about six percent, varying slightly from one run of plants to another. It seemed desirable to know if quantitative demethylation was effected, so demethylation of vanillin, a compound of known methoxyl content, was performed. This produced a 95 percent recovery of the methoxyl group as the quaternary compound under the conditions used. Assuming that a similar situation maintained in lignin, this figure was used as a correction factor in subsequent determinations.

Determination of Radioactivity

All counts were made using a Model 163 Scaling Unit manufactured by Nuclear Instrument and Chemical Corporation. By referring to a self-absorption curve prepared for the substance being counted, all counts made were corrected to zero sample thickness. The samples were counted on aluminum discs 2.83 square cm. in area. The disc containing the sample was placed on the top shelf of the counter assembly of the Scaler Unit. Based on the measured activity of a standard C^{14} sample of known activity, the over-all efficiency was found to be 8.79 percent.

RESULTS

RESULTS

Experiments Using D,L-Methionine

It has been known for some time that methionine can act as a methyl donor in the animal (1). Keller and co-workers fed methionine which was doubly labelled in the methyl group with carbon-14 and deuterium to rats and isolated choline and creatine from the rat tissues (16). Within experimental error, the ratio of deuterium to carbon-14 in methyl groups of choline and creatine was found to be the same as that in the methyl group of the dietary methionine, proving that methionine acts as a true transmethylating agent. Brown has shown that the carbon of the methyl group of methionine can be transferred to the methyl group of nicotine in the tobacco plant. Thus it appeared reasonable that methyl-labelled methionine could give rise to the methoxyl groups of lignin, and it was decided to feed this compound to the plants.

An attempt was made to feed methionine to the plant by immersing the roots in a nutrient solution containing methionine, thus allowing absorption to take place through the roots. Analysis of the solution was necessary to determine the rate of uptake of methionine by the plant and also whether destruction of the methionine by bacteria was taking place. The colorimetric method of analysis used measured the red color produced when methionine reacts with nitroprusside, as described by McCarthy and Sullivan (17).

Each of six barley plants was fed two mg. of methionine in 25 ml. of nutrient solution which contained three drops of the one percent detergent

germicide solution. After forty-eight hours the plants were removed from the solution and analysis for methionine was made. It was found that in two days the average uptake per plant of methionine from solution was 70 percent of the two mg. originally present. No bacterial growth was observed in the medium within this time. As a further check of bacterial activity, samples of the nutrient medium were inoculated with root fragments. After forty-eight hours no decrease was noted in the amount of methionine present, compared with the uninoculated controls.

Plants which had been prepared in the manner previously described were fed radioactive methionine. Each flask contained 2.01×10^{-5} moles of methionine possessing a total activity of 3.6×10^{5} counts per minute (c.p.m.) and three drops of the one percent detergent germicide solution in 25 ml. of nutrient solution. The plants were grown in the specially designed hood under artificial light at room temperature for seven days, as mentioned earlier. At the end of this time no bacterial growth was observable. As a further test of methionine uptake by the plants, replicate samples of the nutrient solution were evaporated to dryness after removing the plants at the end of seven days. Only a small amount of radioactivity was detectable in the residue, indicating almost complete absorption of methionine by the plants.

The roots of the plants were removed and the remaining part of the plants given the previously described solvent extraction suggested by MacDougall and De Long. Lignin was isolated from the remaining fiber by the 72 percent sulfuric acid method. After hydrolysis, filtration and washing, the lignin obtained possessed considerable radioactivity as

determined with a laboratory monitor. In order to bring the radioactivity of the lignin into a range which could be counted with the Scaler Unit, it was necessary to dilute the radioactive lignin with from six to ten times its own weight of inactive lignin obtained from similar plants not fed radioactive material. A weighed amount of about 60 mg. of lignin was placed on a counting disc and counted as outlined previously.

The same sample of lignin used for counting was used for demethylation. Radioactivity was found in the quaternary compound obtained from demethylation of the lignin. Since lignin is not a chemical compound, the specific activity could not be expressed, as is customary, on a molar or millimolar basis. Hence it became necessary to express activity on a recovery of counts basis; that is, by a comparison of the total activity of the quaternary compound obtained from a certain amount of lignin with the total activity of the lignin used. A sample calculation is shown in the Appendix. Recovery of counts obtained from lignin isolated in two experiments is shown in Table II.

TABLE II

METHIONINE EXPERIMENTS

Experiment No.	Observed Activity (counts per minute)		Percent Recovery of Counts
	Lignin	Quaternary Iodide	
1 (24 plants)	637	989	99.0
	467	662	92.9
2 (24 plants)	710	979	90.5
	356	884	89.6

These data show that practically all of the activity found in the lignin is recovered in the quaternary compound obtained upon demethylation under the conditions used. This would indicate that the activity in the lignin is located in the methoxyl group. Variation in the percentage recovery between experiments may be noted. It was generally found in these experiments that recovery of counts from lignin isolated from plants grown at lower temperature (20° C.) was larger than that from plants grown at higher temperature (25-30° C.), but no explanation for this observation is apparent.

Oxidative Degradation Products of Lignin

Lignin isolated from barley plants by the usual procedure was analyzed for nitrogen, using the micro-Kjeldahl method. The nitrogen content was about 2.4 percent, a value lower than that obtained from most lignin preparations. However, the possibility existed that this nitrogen was present in the form of protein and that the radioactivity observed in the lignin was due to the presence of methionine in the protein. To determine definitely whether or not the activity found was associated with nitrogenous compounds, an attempt was made to isolate vanillin, syringaldehyde and similar nitrogen-free derivatives formed by oxidation of lignin with alkaline nitrobenzene, and to test them for radioactivity.

To carry out this reaction, about 145 mg. of lignin isolated from plants fed radioactive methionine were placed in a stainless steel bomb with one ml. of nitrobenzene and 18 ml. of 2N NaOH (20). This was heated at 180° C., with agitation, for about three hours. After cooling, the

alkaline reaction mixture was steam distilled to remove nitrogenous products. The filtrate was acidified to pH 3 and the resulting mixture continuously extracted with benzene for 48 hours. The benzene solution was extracted ten times with 15 ml. portions of aqueous five percent $NaHSO_3$ solution. The bisulfite extraction was acidified with three ml. of concentrated sulfuric acid and the solution freed from sulfur dioxide under reduced pressure at room temperature (21). This solution was then extracted six times with 15 ml. portions of ethyl ether and the ether was then evaporated at 60° C. (22). The residue was extracted with hot water, filtered, and made up to 30 ml. Duplicate 10 ml. aliquots of this solution were used for micro-Kjeldahl nitrogen determinations. Within the experimental error of the determination no nitrogen was found. The aldehyde mixture was precipitated from the rest of the solution using 2,4-dinitrophenylhydrazine. The precipitate was allowed to stand overnight, filtered, dried and counted. The phenylhydrazone mixture possessed a specific activity of 88 c.p.m. per mg. Calculated as vanillin, this would give a specific activity of 192 c.p.m. per mg. The specific activity of the lignin used for oxidation by nitrobenzene was also about 190 c.p.m. per mg.

Experiments Using Formate

Recent experiments show that formate, or a one-carbon compound designated as "formate" appears to play a role in transmethylation reactions. There are two possible ways in which this compound could enter such reactions. Formate may arise from the oxidation of methyl groups and in some manner act directly as a methylating agent, or it may be

reduced to form a methyl group, which is then transferred. Siekevitz, et al.(18), have shown that, in the animal, the methyl group of methionine may give rise to formate. On the other hand, according to Welch and Sakami (19), formate can be reduced to form the methyl group of methionine in the animal organism.

Two groups of plants, as closely similar as possible, were grown in nutrient solution under the same conditions, one group being fed methionine and the other formate. By comparing the activities of lignin isolated from the two groups of plants it was hoped to obtain evidence as to whether methionine or formate acts as the more direct precursor of the methoxyl groups of lignin.

No analytical method could be found which would give accuracy comparable to that achieved in the method used for the determination of methionine. Upon removal of the plants from the nutrient solution after the seven day period, the contents of several of the flasks were evaporated to dryness. Negligible radioactivity was found in the residues. No bacterial growth was observed in the flasks, even after seven days. It was concluded that practically complete absorption of formate by the plants had taken place within this time.

The plants were fed formate under the same conditions that were maintained in the methionine experiments. The flasks each contained the nutrient solution, detergent germicide and 1.99×10^{-5} moles of formate, having a total activity of 7.2×10^{5} c.p.m. The molar concentration was the same as that of methionine--the activity double. As before, the plants were allowed to grow in the solution for seven days.

As previously described, the plants were processed and lignin isolated by the 70 percent sulfuric acid method. The radioactive lignin obtained was counted and demethylated, and the quaternary compound formed was counted. The results obtained using two groups of plants are shown in Table III.

TABLE III

FORMATE EXPERIMENTS

Experiment No.	Observed Activity (counts per minute)		Percent Recovery of Counts
	Lignin	Quaternary Iodide	
1 (25 plants)	243	283	71.7
	241	256	69.8
2 (25 plants)	329	412	66.8
	181	212	64.8

It can be seen that most of the activity found in the lignin was recovered by demethylation, showing that it was present in the methoxyl group. The variation between the two runs reported here may again be due to difference in temperature at which the plants were grown, although this was smaller here than in the methionine experiments.

As stated earlier, a comparison of the activities of lignin obtained from plants fed methionine and that from plants fed formate was made. Total activities, molar quantities administered per plant and the specific activities of the lignin isolated from each group of plants are given in Table IV. The specific activity of lignin obtained after administering

TABLE IV

COMPARISON OF ACTIVITIES OF LIGNIN FROM PLANTS FED METHIONINE AND THOSE FED FORMATE

Group No.	Compound Administered	Moles Given per Plant	Total Activity Given per Plant	Specific Activity of Lignin
1	Methionine	2.01×10^{-5}	3.6×10^{5} c.p.m.	385 c.p.m./mg.
2	Formate	1.99×10^{-5}	7.2×10^{5} c.p.m.	29.2 c.p.m./mg.

methionine was about thirteen times as high as that obtained in the formate experiment. The molar concentrations of methionine and formate administered were the same, but the activity of the formate was twice as great. Considering this, it appears that if C^{14}- methyl methionine is fed to barley plants, the specific activity of the lignin isolated is at least one order of magnitude higher than that obtained after feeding C^{14}-formate, all conditions being kept as nearly the same as possible in the experiments.

DISCUSSION

DISCUSSION

It is demonstrated in these experiments that the methyl carbon of methionine can act as the precursor of the methoxyl carbon of lignin produced by barley plants *in vivo*. The results obtained give a strong indication that a transmethylation reaction is involved. In order to prove conclusively that such a reaction has occurred, i.e., that the methyl group is transferred intact, it will be necessary to conduct experiments using methionine doubly labelled with carbon-14 and deuterium in the methyl group. Such experiments will soon be undertaken in this laboratory. If a true transmethylation is proven, the first instance of transmethylation to an oxygen atom will be established.

Formate can also act as precursor of the methoxyl group of lignin but to a lesser extent than the methyl group of methionine. The lower specific activity of the lignin obtained from plants fed formate as compared with those fed methionine indicates that the methyl group of methionine is transferred as such and does not go through a process of oxidation to formate and subsequent reduction. If the latter were true, the lignin from plants fed formate would have at least as high a specific activity as that from plants fed methionine. From these results it would also appear that formate is not transferred as such and then reduced. Although Welch and Sakami found that formate may be reduced to the methyl group of methionine, the exact manner in which this takes place is not known, but there is some evidence that the reaction takes place via serine, ethanolamine, choline and betaine (23). It is conceivable that

choline and betaine act as methylating agents in the formation of lignin, methionine not necessarily being formed before transmethylation takes place. While most of the formate present in the plant probably is oxidized to carbon dioxide, it seems possible that some could be reduced to methyl groups and involved in methylation reactions.

It is not known why, in demethylation of lignin from plants fed formate, a lower recovery of counts is obtained than from lignin formed by plants fed methionine. In several theories of lignin formation carbohydrates are named as lignin precursors. Sakami (23) has shown that formate and glycine, through serine, form pyruvate. This pyruvate could give rise to carbohydrates, from which lignin might conceivably be formed. The lignin formed would very likely possess radioactivity from the formate in positions other than methoxyl groups, subsequent demethylation of the lignin giving a lower recovery of radioactivity. This is a possible explanation of the low results obtained.

Elwyn and co-workers found that in the synthesis of methyl groups from serine the β-carbon does not go through the oxidation level of formate. The β-carbon of serine has an oxidation state midway between that of formate and a methyl group. Hence it might be expected, if the labile methyl group were to be oxidized to this stage and then reduced again to a methyl group, that the specific activities of lignin from plants fed formate and those fed methionine would be of about the same order. Since the difference in specific activities is so large, it would seem logical that the methyl group is transferred without this oxidation and reduction. This aspect could be further investigated by feeding the

plants labelled serine and comparing the activity of the lignin from these plants with that from plants in the formate and methionine experiments.

In such a comparison as that made between lignin from plants fed methionine and formate, several complications may arise. In order that a fair comparison can be made it is necessary, among other factors, that the rates of uptake of chemicals fed be about the same and that the size of the pools of these chemicals in the plant remains rather constant. It is possible that a variation in these factors could appreciably affect the results and conclusions. However, the conditions were kept as constant as possible, and since a difference in specific activities of more than one order of magnitude was observed, these conclusions appear to be valid.

The results obtained in the present study differ greatly from those of Kirkwood and Marion. They found that radioactive formate fed to barley plants was appreciably incorporated into the methyl groups of hordenine, but that radioactive methyl groups of choline fed to the plants were not. In this laboratory Wing (24) found that, in 24 hours, an extensive destruction of choline in nutrient solution inoculated with root fragments had taken place, indicating bacterial activity. Since the above authors make no mention of precautions taken against bacterial growth, it seems probable that the choline was destroyed by bacterial action before it could be absorbed by the plants.

That the radioactivity was present in the methoxyl group and not in methionine or any other nitrogenous component was proved by precipitating

aldehydes formed upon oxidation of lignin as 2,4-dinitrophenylhydrazones from a nitrogen-free solution. For a convenient basis of comparison, the activity found in the phenylhydrazone mixture was calculated as specific activity of vanillin and this was found to be on the same order as that of the lignin which was oxidized. If much of the radioactivity in the lignin were associated with the nitrogen present, the specific activity of the vanillin would be appreciably lower than that of the lignin used for oxidation.

The results of the present study give evidence that in H. vulgare transmethylation is involved in formation of lignin. Although there is evidence to disprove Zherebov's statement that lignification is merely an accumulation of methoxyl groups or a process of methylation (25), it is probably true that most theories of lignin formation do not place due emphasis upon the importance of the role transmethylation may play in the process. Evidence for direct methylation appears to be more convincing than that supporting Klason's theory that formaldehyde acts as the precursor of methoxyl groups. Before the process of lignin formation is understood thoroughly a great deal more experimental work is necessary. More light could probably be shed on the problem by feeding plants carbohydrates labelled in the carbon chain and/or in methoxyl groups. Determination of the location of radioactivity in lignin synthesized by these plants would give a further indication of the manner in which the lignin is produced.

SUMMARY

SUMMARY

1. Radioactive methionine was administered to barley plants. Lignin isolated from these plants possessed radioactivity. Degradation showed that practically all of this activity was located in the methoxyl groups.

2. Lignin with a lower level of radioactivity was also isolated from barley plants which had been fed radioactive formate. Most of this activity was found in the methoxyl position.

3. On the basis of these results it is postulated that the methyl group is transferred as an entity, without oxidation and reduction taking place. It is further postulated that formate, as such, is not transferred, but that reduction to a methyl group is first undergone.

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APPENDIX

APPENDIX

The following formula was used for the calculation of percentage recovery of counts:

$$\% \text{ recovery} = \frac{C_o \text{ (quat.)}/a \quad X \quad 100}{C_o \text{ (lignin)}/b \quad X \quad 95} \times 100,$$

where C_o (quat.) = observed count of quaternary compound (c.p.m.)

a = fraction of maximum specific activity at sample thickness used--from self-absorption curve for quaternary compound,

C_o (lignin) = observed count of lignin (c.p.m.),

b = fraction of maximum specific activity at sample thickness used--from self-absorption curve for lignin,

$\frac{100}{95}$ = correction factor, based on 95% recovery of methoxyl groups from vanillin.

Sample calculation:

C_o (quat.) = 741 c.p.m.

a = .584

C_o (lignin) = 467 c.p.m.

b = .325

$$\% \text{ recovery} = \frac{741/.584 \quad x \quad 100}{467/.325 \quad x \quad 95} \times 100 = 92.9\%$$