THE INCORPORATION OF C^{14}-LABELED PHENYLALANINE, TYROSINE, AND GLUCOSE INTO CORN ROOTS[1, 2]

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ABSTRACT

Corn plants given C^{14} uniformly labeled phenylalanine, tyrosine and glucose, incorporated phenylalanine at the most rapid rate. The lignin fraction of the plant was found to have the greatest activity, especially in phenylalanine-labeled plants.

Studies on the breakdown of lignins into humic substances have been hindered because of the lack of knowledge of lignin and its formation in plants. Both glucose and shikimic acids have been shown to be incorporated into plant lignins by Eberhardt and Schubert (1956). The number of methoxy groups on the lignin molecule increases with the age of the plant lignin and is one of the parts of the lignin molecule to be attacked by soil micro-organisms (Broadbent, 1954). The manner in which molecules of phenolic origin are arranged in the lignin is of great importance in understanding its breakdown in the soil. The purpose of the experiment was to see if it is possible to obtain plant roots with high radioactivity in the lignin fraction and to study the formation of this molecule.

METHODS AND MATERIALS

To a series of plants was given a 0.1 mc of uniformly labeled C^{14} D-glucose, and to another series 0.1 mc of uniformly labeled C^{14} L-phenylalanine. The corn plants were two weeks old and grown in sand cultures. The isotopes were dissolved into nutrient solutions and fed to the plants daily over a 10-day period. The solutions containing the isotopes were kept under refrigeration to prevent decay by micro-organisms. The plants were harvested, dried, and ground. The lignin was extracted by the Klason or 72 per cent sulfuric acid method. These plant materials were counted by a thin-end-window gieger counter, using uniform planchets and sample material. The lignin was degraded by thermal decomposition and the phenols were isolated by chromatography as the diazotized phenols (Hossfeld, 1951). Lignin samples were hydrolyzed with 4 per cent HCl and the amino acids were separated by paper chromatography using butanol-formic acid-water (150–30–20). The amino acids were identified as ninhydrin-reacting compounds and compared to spots of known amino acids separated under the same conditions. Nitrogen content was determined by micro-Kjeldahl and the methoxy content of the lignin was determined by the Zeisel method (Smith and Shriner, 1956).

The isolated spots on the paper chromatograms were then analyzed for radioactivity by cutting out the spots and counted with a model 701 Nuclear Chicago scintillation counter. The composition of the scintillation liquid is as follows:

C. P. toluene 1.0 liter; PPO 4.0 g; POPOP 0.100 g

The above experiment was repeated (Experiment II), using uniformly labeled C^{14} phenylalanine and C^{14} tyrosine. Tyrosine was used as a precursor to minimize any decomposition that could have occurred to glucose during the feeding period. The plants in table 3 were slightly older (21 days) and were fed over a shorter period of time (7 days). The methods of analysis were the same as those listed above.

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RESULTS AND DISCUSSION

Of the three C^{14} starting materials, (glucose, tyrosine, and phenylalanine) phenylalanine was incorporated into the lignin fraction of corn roots to the greatest extent (tables 1 and 3). Hydrolysis of the lignin fraction (isolated by 72 per cent sulfuric acid) consistently gave amino acids associated with the lignin as shown in tables 2 and 4. The amino acid with the highest activity was phenylalanine. Acerbo, Nord, and Schubert (1957) used p-hydroxy phenylpyruvic acid as a precursor in studies with lignins and found a large activity when the fraction was degraded to $BaCO_3$. Decomposition of the lignin fraction to $BaCO_3$ as a means to determine radioactivity in the lignin fraction would give erroneous results if aromatic precursors were used. In this work there can be no positive idea of how much activity is coming from the lignin molecule or the phenylalanine attached to this fraction. Certainly both have been shown to possess radioactivity.

EXPERIMENT I

TABLE 1

Distribution of activity in corn roots, tops, and lignin from D-glucose-C^{14} and L-phenylalanine-C^{14}

	Roots counts min^{-1} g^{-1}		Tops counts min^{-1} g^{-1}	
Glucose	170		62	
Phenylalanine	506		110	
	Lignin *counts min^{-1} g^{-1}	% yield	N content %	Methoxy %
Glucose	203	21.4	1.82	5.82
Phenylalanine	1006	22.8	2.18	5.72

*0.3 g of lignin used for counts.

TABLE 2

Activity of various phenols, tyrosine and amino acids isolated from phenylalanine labeled roots

	L-Phenylalanine-C^{14} counts min^{-1}
Phenol	93
Guaiacol	26
2,6-dimethoxy phenol	34
Hydrolysis of lignin	L-Phenylalanine-C^{14} counts min^{-1}
Phenylalanine	223
*Unknown	69
Tyrosine	47
Glycine	25
Alanine	14

No appreciable activity was found in the D-glucose-C^{14} samples. All samples were counted with model 701 N.C. liquid scintillation counter.

*A spot in this area could be leucine or methionine.

EXPERIMENT II

TABLE 3

Radioactivity of various compounds in plant roots labeled with C^{14} phenylalanine and tyrosine

	Roots counts min^{-1} g^{-1}	Tops counts min^{-1} g^{-1}
Phenylalanine	3,104	629
Tyrosine	1,977	124
	Lignin counts min^{-1}	% yield
Phenylalanine	4160[1]	35
Tyrosine	2739[2]	29

[1]0.55 g of lignin used for counting.
[2]0.65 g of lignin used for counting.

TABLE 4

Activity of various phenols, tyrosine, and amino acids isolated from phenylalanine-labeled lignins[1]

	L-Phenylalanine-C^{14} counts min^{-1}
Phenol	347
Guaiacol	213
2,6-dimethoxy phenol	116

Hydrolysis of lignin

	Phenylalanine counts min^{-1}	Tyrosine counts min^{-1}
Phenylalanine	196.0	429
Glutomic-aspartic acids	*none	—
Glycine	none	—
Tyrosine	86.6	49
Alanine	79.0	—

*Amount less than control.
[1]Counts were made on spots taken from the chromatograms.

Degradation of the lignin fraction to aromatic phenols showed that phenol had a higher activity than guaiacol or 2,6-dimethoxy phenol in C^{14}-labeled phenylalanine roots. The sequence in the formation of the lignin molecule is of great importance to studies on its decomposition. The question is, is phenol formed first, then merely methylated to form the other units, or do the aromatic nuclei form first and then become incorporated in the polymer? A decrease in methoxy content is one of the first steps known to occur in the decomposition of lignin to humic acids in the soil. The availability of these methoxy groups to microbiological decomposition would be influenced by their formation in the lignin molecule and the consequent composition of the decomposing lignin.

In this work no attempt was made to correct for self-absorption because of the rather small amount of root material obtained. The samples were merely compared by counting equal amounts of plant material and comparing the amounts of radioactivity. Certainly the counting rates for glucose and phenylalanine, and phenylalanine and tyrosine lignins are great enough that it can be concluded that phenylalanine is incorporated into the lignin fraction to the greatest extent. The

radioactivity of phenol in the lignin fraction is greater than either of the other two phenols isolated (Counting rates are given not as totals, but as amounts above background counts). Glucose-labeled roots showed little activity in the lignin fraction or in the hydrolysate of the lignin molecules. Present studies are being continued with sterile root systems, which would eliminate any possibility of microbial decomposition before incorporation into the root. It now seems possible to obtain a plant sample with a high degree of activity in the lignin fraction.

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