A STUDY OF THE BIOSYNTHESIS OF THE METHOXYL GROUPS OF LIGNIN IN TOBACCO PLANTS*

BY ROBERT L. HAMILL,† RICHARD U. BYERRUAM, AND CHARLES D. BALL

(From the Kedzie Chemical Laboratory, Michigan State University, East Lansing, Michigan)

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Previous studies in our laboratory have been concerned with the pathway of carbon in the formation of methyl groups in photosynthesizing plants. In the biosynthesis of the N-methyl group of nicotine in tobacco plants, it was shown that, of several possible precursors, formaldehyde was incorporated to the greatest extent (1). This finding led to the suggestion that a "1-carbon" unit at the oxidation state of formaldehyde was reduced to methyl groups in various plant products. It was further shown that the α-carbons of glycine1 and glycolate and the β-carbon of serine were also incorporated into the N-methyl group of nicotine to a relatively large extent, although to a lesser extent than was formaldehyde (1-3). These results were interpreted to indicate that, in the formation of the N-methyl group, a 2- or 3-carbon compound is decomposed to yield the "1-carbon" unit at the oxidation state of formaldehyde.

The biosynthesis of O-methyl groups, which occurs in plants but not in animals, likewise has received attention. The O-methyl group of the alkaloid, ricinine, synthesized by etiolated castor bean seedlings, was shown to arise from the methyl group of methionine. However, formate did not appear to be reduced to methyl groups in these plants (4). In growing, photosynthesizing barley and tobacco, the O-methyl groups of the cell wall constituent lignin were shown to arise either by transmethylation from methionine or by reduction of formate or of the α-carbon of glycolate (3, 5). Formate was incorporated to a relatively small extent in comparison with methionine or glycolate. It was therefore of interest to study some other possible precursors of the O-methyl group which might occur in the metabolic pathway from carbon dioxide.

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† Present address, Eli Lilly and Company, Indianapolis, Indiana.

1 The labeled glycine was purchased from Tracerlab, Inc., Boston, Massachusetts, the nL-serine-3-C14 from the California Foundation for Biochemical Research, Los Angeles, and the formaldehyde-C14 from Isotopes Specialties Company, Inc., Glendale, California.

713
BIOSYNTHESIS OF LIGNIN METHOXYL GROUPS

EXPERIMENTAL

Glycine-1-C\textsuperscript{14}, glycine-2-C\textsuperscript{14}, dl-serine-C\textsuperscript{14}, and formaldehyde-C\textsuperscript{14} were fed in a nutrient solution to separate groups of tobacco plants (Nicotiana rustica L.) as previously described (6). These compounds (1) have been shown to be readily absorbed by the plants through the roots. Each plant received 1.3 to 1.6 $\times$ 10\textsuperscript{-5} mole of the radioactive compound with a radioactivity of 1 $\times$ 10\textsuperscript{5} c.p.m. After a 7 day growing period, lignin was isolated from each group of plants by the procedure of Ost and Wilkening (7) as modified by MacDougal and DeLong (8), and measured for radioactivity with a thin end window Geiger-Müller tube which had an efficiency of 2 per cent. The radioactivity present in methoxyl groups was ascertained by demethylation of the lignin by a modification of the method of Phillips in which the methyl iodide resulting from cleavage of the methoxyl groups with hydriodic acid was allowed to react with triethylamine to give methyltriethylammonium iodide (5). This compound was counted for radioactivity.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comparison of Various Compounds As Precursors of Methoxyl Groups in Tobacco Lignin</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound fed</th>
<th>Lignin, c.p.m. per 60 mg.</th>
<th>Methyltriethylammonium iodide, c.p.m. recovered from 60 mg. lignin</th>
<th>Per cent recovery of counts upon demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine-3-C\textsuperscript{14}</td>
<td>8100</td>
<td>7240</td>
<td>90</td>
</tr>
<tr>
<td>&quot;</td>
<td>5360</td>
<td>4660</td>
<td>87</td>
</tr>
<tr>
<td>Formaldehyde-C\textsuperscript{14}</td>
<td>4415</td>
<td>4350</td>
<td>99</td>
</tr>
<tr>
<td>&quot;</td>
<td>4020</td>
<td>3330</td>
<td>98</td>
</tr>
<tr>
<td>Glycine-2-C\textsuperscript{14}</td>
<td>1830</td>
<td>1600</td>
<td>88</td>
</tr>
<tr>
<td>&quot;</td>
<td>1650</td>
<td>1527</td>
<td>93</td>
</tr>
<tr>
<td>Glycine-1-C\textsuperscript{14}</td>
<td>716</td>
<td>23</td>
<td>3.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>675</td>
<td>11</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Results

The radioactivity of lignin isolated from different groups of plants fed glycine-1-C\textsuperscript{14}, glycine-2-C\textsuperscript{14}, serine-3-C\textsuperscript{14}, and formaldehyde-C\textsuperscript{14} is presented in Table I. Since the molecular formula for lignin is unknown, its radioactivity was expressed as counts per minute per 60 mg. of lignin, this being a convenient quantity for counting. The counts, in all cases, were corrected for self-absorption.

It may be seen that the $\beta$-carbon of serine is introduced into lignin to the greatest extent, and formaldehyde to a slightly smaller extent. The
\(\alpha\)-carbon of glycine was incorporated in about one-third the quantity of the \(\beta\)-carbon of serine, whereas the glycine carboxyl group was introduced in about one-tenth the quantity of the \(\beta\)-carbon of serine. Variations in the radioactivity of lignin, after serine-3-\(\text{C}^{14}\) was fed to different groups of plants, were probably due to differences in seasonal rate of growth and metabolism.

After feeding serine-3-\(\text{C}^{14}\), glycine-2-\(\text{C}^{14}\), and formaldehyde-\(\text{C}^{14}\), the majority of the radioactivity of the lignin could be recovered upon demethylation (Table I). However, demethylation of lignin isolated from plants fed glycine-1-\(\text{C}^{14}\) resulted in a recovery of only about 2 per cent of the radioactivity. Analysis of lignin for total carbon and for methoxyl carbon showed that about 2 to 3 per cent of the carbon in lignin is in the methoxyl group.

**DISCUSSION**

Previous studies of metabolism in tobacco plants have shown that, of several methyl group precursors, formaldehyde enters nicotine to the greatest extent, followed by the \(\alpha\)-carbon of glycine and the \(\beta\)-carbon of serine (1, 2). Because of the difference in relative degree of incorporation of serine into the \(N\)-methyl and \(O\)-methyl groups, as reported in the present study, the mechanism of formation of the nicotine methyl group and lignin methoxyl group would appear to be somewhat different, particularly where serine is a precursor.

It was also suggested previously (2) that glycine might supply its nitrogen to form the pyrrolidine ring nitrogen of nicotine and that its \(\alpha\)-carbon might become the methyl group without separation of the 2 atoms. In studying the biosynthesis of lignin, it is found that the \(\alpha\)-carbon of glycine enters the methoxyl group to a relatively large extent. Since the \(\alpha\)-carbon of glycine must be separated from the amino nitrogen to form a methoxyl group, it seems reasonable to postulate that a separation of carbon from nitrogen to yield a 1-carbon unit from glycine occurs in the synthesis of both nicotine and lignin.

Because of the relatively large incorporation of the \(\beta\)-carbon of serine, the \(\alpha\)-carbon of glycine and formaldehyde into methyl groups, it is possible that these compounds may either be in, or closely related to, compounds in the pathway of conversion of carbon dioxide to methyl groups. The work of Wilson and Calvin (9) and of Stumpf and Horecker (10) suggests that glycolic acid, which was shown to be a methyl group precursor (3), may be formed from the 1 and 2 carbons of xylulose-5-phosphate, derived from ribulose-5-phosphate, by hydrolysis of the carbohydrate in the presence of transketolase. Schou et al. (11) and Tulbert and Cohan (12) have indicated that glycolic acid, glycine, and serine are all
interconvertible. The carbons of glycolic acid give rise to the corresponding carbons of glycine by oxidation to glyoxylic acid and subsequent transamination. Glycolic acid also may give rise to serine, the carboxyl and \( \alpha \)-carbon of the serine arising from the corresponding carbons of glycolic acid, and the \( \beta \)-carbon of serine arising from the \( \alpha \)-carbon of glycolic acid through a 1-carbon intermediate.

Therefore, one possible mechanism for synthesis of methyl groups in photosynthesis is through ribulose phosphate and xylulose phosphate to give a 2-carbon compound, glycolate, which may be converted to glycine and serine. The \( \alpha \)-carbons of glycolate or glycine and the \( \beta \)-carbon of serine can then yield a 1-carbon unit, at the oxidation state of formaldehyde, which is reducible to methyl groups (13).

**SUMMARY**

The labeled carbons of glycine-1-\( ^{14} \text{C} \), glycine-2-\( ^{14} \text{C} \), serine-3-\( ^{14} \text{C} \), and formaldehyde-\( ^{14} \text{C} \) were incorporated into lignin in tobacco plant metabolism. About 90 per cent of the radioactivity of the lignin was found to be in the methoxyl carbon after feeding glycine-2-\( ^{14} \text{C} \), serine-3-\( ^{14} \text{C} \), and formaldehyde-\( ^{14} \text{C} \), whereas only 2 to 3 per cent of the radioactivity of lignin was present in the methoxyl carbon after feeding glycine-1-\( ^{14} \text{C} \). When these possible methyl group precursors were fed under similar conditions, serine-3-\( ^{14} \text{C} \) was incorporated into lignin to the greatest extent, followed in order by formaldehyde-\( ^{14} \text{C} \), glycine-2-\( ^{14} \text{C} \), and glycine-1-\( ^{14} \text{C} \).

**BIBLIOGRAPHY**

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