Biosynthesis of the Allylphenols Eugenol and Methyleugenol in
Ocimum basilicum L.

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Summary The allylphenols eugenol and methyleugenol are synthesized in O. basilicum from ferulic acid and coniferyl alcohol whose sidechains are converted into allyl groups with retention of all carbon atoms, and hydrogen atoms of the hydroxy-group are quantitatively incorporated into the allyl group; a previous hypothesis for the biosynthesis of these compounds, which involved loss of the C-1 from these precursors, has therefore to be rejected.

On the basis of comparative phytochemical considerations it can be assumed that allyl- and propenyl-phenols in higher plants originate from the cinnamate carbon skeleton. The most likely intermediates are substituted cinnamyl aldehydes and cinnamyl alcohols. However, recent studies on the biosynthesis of eugenol in Ocimum basilicum led Canonica et al. to reject this possibility. They fed D,L-phenylalanine, labelled with 14C in different positions of the side chain mixed with non-specifically tritiated L-phenylalanine and observed in the case of [1-14C]phenylalanine an increase in the 3H:14C ratio of the isolated eugenol. This was interpreted as being due to loss of the carboxyl group of ferulic acid, incorporation of an 'extra' carbon atom, and removal of the hydrogen atoms from C-1 of coniferyl alcohol did not occur during the biosynthesis of eugenol and methyleugenol.

A high-eugenol-yielding O. basilicum strain 'Genovese' (Ingegnoli, Milan, Italy) was used for these experiments. Glucoferulic acid was synthesized by a Knoevenagel reaction of [1-14C]malonic acid with acetylated [O-CH3]glucovanillin. Thus the labelled ferulic acid unit contained 14C and 3H within the same molecular species. [14C; 3H]-Coniferin was synthesized by standard procedure from the above glucoferulic acid. [γ-14C3H3OH]Coniferin was syn-

\[\text{Ar-CH} \cdot \text{CH-CO}_2\text{H} \rightarrow \text{Ar-CH} \cdot \text{CH-CHO} \rightarrow \text{Ar-CH} \cdot \text{CH}_2\text{OH} \]

\[\text{Ar-CH}_2\text{-CCH}_2\]

\[\text{Ar-CH} \cdot \text{CH-Me}\]

Scheme
TABLE. Incorporation of potential precursors into allylphenols.

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<tbody>
<tr>
<td>Glucoferulic acid</td>
<td>1.27</td>
<td>21.89; 2.15</td>
<td>10.18</td>
<td>10.16</td>
<td>10.31</td>
<td>0.04</td>
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<tr>
<td>[γ-14CO2H; -O-CH₃]</td>
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<tr>
<td>Glucoconiferyl alcohol</td>
<td>1.00</td>
<td>24.90; 2.20</td>
<td>11.31</td>
<td>0.012</td>
<td>9.22</td>
<td>0.015</td>
</tr>
<tr>
<td>[γ-14CH₃OH; -O-CH₃]</td>
<td>0.54</td>
<td>18.67; 1.73</td>
<td>10.79</td>
<td>0.016</td>
<td>10.87</td>
<td>1.0</td>
</tr>
<tr>
<td>[γ-14CH₂OH]</td>
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</table>

* The precursors were fed through the cut ends of two *O. basilicum* plants (11 g per plant). After 2 h the plants were steam distilled. Their identity and purity was checked by radio g.l.c. and m.s. On an average, 16 μmol of eugenol and 54 μmol of methyleugenol was isolated. * Average of 4 independent feeding experiments. * Average of 5 independent feeding experiments.

thesized by reduction of acetylated [γ-14COCl]glucoferuloyl chloride with LiB₃H₄. Tritium was exclusively located in the alcohol group of coniferin, as 53% (calc. 50%) of tritium was lost when coniferyl alcohol liberated by the action of β-glucosidase from coniferin was enzymatically converted into coniferyl aldehyde by means of specific cinnamyl alcohol dehydrogenase (NADP). The same results were obtained when [γ-14CH₂OH]coniferin was oxidised with MnO₂ to give the corresponding aldehyde. Glucose derivatives were used in feeding experiments because they are more stable and soluble compared to the free phenols. The glucose part of the precursors is believed to split off during the passage through the plasmalemma of the plant cells. Experiments using the three differently labelled precursors showed that in all cases incorporation of radioactivity into the allylphenol derivatives eugenol and methyleugenol of *O. basilicum* occurred (Table).

A comparison of the H:14C ratio of precursor and product clearly shows that there is no change in this ratio and therefore no loss of the C-1 atom of the sidechain during the in vivo conversion, as has been suggested earlier. Unequivocal degradation of the terminal carbon atom of both allylphenols from a ferulic acid feeding experiment demonstrated that ca. 97% of the 14C-label was located in this terminal position, corresponding to C-1 of the precursors, since the resulting homoveratrum aldehyde contained only tritium and was devoid of 14C-activity. Of considerable interest is the fact that [γ-14CH₃OH]coniferin is incorporated into eugenol without change of the H:14C ratio. This demonstrates that both hydrogen atoms, linked directly to C-1 of the cinnamyl alcohol, are retained during the reduction to allylphenols and proves that these alcohols are immediate precursors in this conversion.

These experiments show that allyl- and propenyl-phenol derivatives of higher plants have common precursors and are biosynthesized as shown in the Scheme. The exact mechanism by which cinnamyl alcohol derivatives are reduced to allyl- or propenyl-phenols has still to be investigated.

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6 J. Stöckigt, unpublished results.