On the mode of incorporation of shikimic acid into 2-hydroxy-1,4-naphthoquinone (lawsone)

M. H. ZENK and E. LEISTNER

Botanisches Institut der Universität München *


Recently CHEN and BÖHM have demonstrated that shikimic acid is incorporated into lawsone (fig. 1) a naphthoquinone in Impatiens balsamina L. This fact is in accordance with experiments which demonstrate the incorporation of this acid in vitamin K₂ and vitamin K₃ in bacteria and higher plants. Degradation of labeled vitamin K₂ after feeding shikimic acid (U-¹⁴C) has shown that the intact ring of this acid is incorporated into the benzenoid moiety of this naphthoquinone. In contrast, the degradation studies on lawsone indicated that the ring atoms of shikimic acid (U-¹⁴C) are transformed into the quinone ring of 2-hydroxy-1,4-naphthoquinone. This assumption was based on the fact, that on oxidative degradation of the labeled lawsone only 68,5% of the radioactivity originally present in the quinone was found in phthalic acid; the result agrees well with the theoretical value of 67% calculated on the basis that the shikimic acid ring is incorporated into carbon atoms 1, 2, 3, 4, 9 and 10 of the naphthoquinone (fig. 1). Oxidation of the quinone to phthalic acid would then result in the loss of carbon atoms 2 and 3 and of 33% of the label. Forced by the interpretation of their results CHEN and BÖHM adopted the hypothesis that p-hydroxybenzoic acid or hydroquinone might be possible precursors of lawsone. They could strengthen their assumption by administration of unlabeled p-hydroxybenzoic acid together with labeled shikimic acid which resulted in no incorporation of ¹⁴C into lawsone; p-hydroxybenzoic acid is known to be a precursor of hydroquinone in higher plants.

There is obviously a discrepancy between the mode of incorporation of shikimic acid in naphthoquinones in bacteria and higher plants and we decided therefore to repeat the work of CHEN and BÖHM. Plants were grown fed, and the lawsone isolated according to I. c. The quinone was purified by thinlayer chromatography to constant specific activity and degraded with alkaline potassium permanganate to phthalic acid which was decarboxylated.

The results obtained (tab. 1) clearly indicate, that the ring atoms of shikimic acid (1,2-¹⁴C) and (U-¹⁴C) are incorporated into the benzene moiety (that is carbon atoms: 5, 6, 7, 8, 9, 10) and not into the quinone ring of the naphthoquinone as suggested previously. Furthermore these results show for the first time, that during naphthoquinone biosynthesis shikimic acid is incorporated in toto, the carboxylgroup (15% ¹⁴C) of the acid being transformed into one, or equally into each, of the keto groups (C-1 and 4) of the quinone ring (found: 15,8% ¹⁴C); this holds true also for the biosynthesis of vitamin K₃. In addition p-hydroxybenzoic acid is not a precursor of the naphthoquinone; the above mentioned competition between shikimic and p-hydroxybenzoic acid must be an indirect effect and can not be explained on the basis of the latter being an intermediate in the formation of naphthoquinones.

Our thanks are due to Mr. K. BOLKART of this laboratory, for the synthesis of ring-¹⁴C-p-hydroxybenzoic acid, to Miss M. KOBLOCH for her excellent technical assistance and to the "Deutsche Forschungsgemeinschaft" for financial support.

Tab. 1. Incorporation of different potential precursors into lawsone and degradation of labeled 2-hydroxy-1,4-naphthoquinone.

<table>
<thead>
<tr>
<th>precursor</th>
<th>material fed** [dpm]</th>
<th>precursor</th>
<th>material fed** [dpm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[μMol]</td>
<td></td>
<td>[μMol]</td>
</tr>
<tr>
<td></td>
<td>* 10⁶</td>
<td></td>
<td>* 10⁶</td>
</tr>
<tr>
<td>DL-shikimic acid (1,2-¹⁴C)</td>
<td>5.33 2.015 0.916*</td>
<td>100800 157000 104 1817 23 2.3</td>
<td></td>
</tr>
<tr>
<td>D-shikimic acid (U-¹⁴C)</td>
<td>3.61 2.77 3.28</td>
<td>325000 314000 96.8 28800 15.8</td>
<td></td>
</tr>
<tr>
<td>p-OH-benzoic acid (ring-¹⁴C)</td>
<td>1.63 3.23 0 0 0 0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calculated on the basis, that only the α-form of shikimic acid is incorporated. ** feeding time: 24 hrs.

2 G. B. COX and F. GIBSON, Biochem. J. 100, 1 [1966].
3 E. LEISTNER and M. H. ZENK, Unpublished results.
8 München 19, Menzinger Straße 67.