Occurrence of trans-3-Hexenal in Thea sinensis Leaves

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trans-3-Hexenal, Tea Leaves, Linolenic Acid

Based on a discovery of trans-3-hexenal and participation of isomerases and non-enzymatic isomerizing factors, the biogenetic pathway of trans-2-hexenal (leaf aldehyde) and cis-3-hexenol (leaf alcohol) has been demonstrated.

Leaf alcohol, cis-3-hexenol, and leaf aldehyde, trans-2-hexenal, are widely distributed in fresh leaves, vegetables and fruits and are responsible for the green odor characteristic of the leaf. Also, both are found in some insect excretions as functioning attractants and repellents [1].

The investigation of leaf alcohol has been carried out by Takei and Ohno et al. since 1938 [2]; from 1957, we have been studying leaf alcohol and leaf aldehyde from a number of angles: synthetic chemistry, natural products chemistry, and plant biochemistry [3].

We have discovered the isomers of leaf alcohol, trans-3-hexenol [4], trans-2-hexenol [5] and cis-3-hexenal [6, 7] in tea leaves and demonstrated that leaf alcohol and leaf aldehyde are produced from cis-3-hexenal, which is biosynthesized from linolenic acid by enzymatic oxygenative splitting between C-12 and C-13 through the addition of oxygen [8, 9].

Leaf alcohol and leaf aldehyde are biosynthesized as shown in Fig. 1, based on a discovery of trans-3-hexenal and participation of isomerase (E4-E6) and non-enzymatic isomerizing factor (IF).

i) trans-3-Hexenal. The geometrical isomer of cis-3-hexenal, trans-3-hexenal, has not been found in nature so far since the βγ-isomer is not separated from cis-3-hexenal on gas chromatography (GC) under the usual conditions [6].

Pilot experiments with GC equipped column treated with AgNO3, using synthetic cis-3-hexenal and trans-3-hexenal [7], had indicated that this procedure is suitable for separation of the cis- and trans-βγ-hexenals as shown in Table I.

Tea leaves homogenates in rubber stoppered flask, was incubated at 35 °C for 10 min and the head space vapor was analyzed by GC. As shown in Fig. 2, trans-3-hexenal was first identified in tea leaves by comparison of retention times and mass spectra of synthetic trans-3-hexenal.

ii) E4-E6 and IF. When tea leaves homogenates was incubated with linolenic acid, cis-3-hexenal was
produced at first and then isomerized to trans-3- and trans-2-hexenal by isomerases (E4-E6). Also, with heat-denatured homogenates, isomerization of cis-3-hexenal to trans-3- and trans-2-hexenal was observed. Thus, it is strongly suggested that IF in addition to E4-E6 participates in the isomerization of C6-aldehydes in tea leaves.

Three grams of fresh tea leaves (July, 1979) were homogenized in a Waring blender for 3 min with 10 ml of McIlvain's buffer, pH 6.3 and 6 μmol of substrates, cis-3-hexenal or trans-3-hexenal. The homogenate was immediately transferred into a 50 ml flask which was then sealed. The reaction mixture was incubated with shaking at 35 °C for 10 min and six ml of head space vapor was quantitatively analyzed by GC (Table II).

Table II indicates that trans-3-hexenal and trans-2-hexenal increased on addition of cis-3-hexenal as substrate and trans-2-hexenal increased on addition of trans-3-hexenal.

These findings suggest that cis-3-hexenal, trans-3-hexenal and trans-2-hexenal are interconverted by regulation with E4-E6 and IF as shown Fig. 1.

However, the details on E4-E6 and IF are in progress.

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**Table I. Gas-chromatography of trans-3-hexenal and cis-3-hexenal.**

<table>
<thead>
<tr>
<th></th>
<th>AgNO3 [％]</th>
<th>H3PO4 [％]</th>
<th>Length [m]</th>
<th>Retention time [min]</th>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>SP 1200 15％*1</td>
<td>5</td>
<td>3</td>
<td>cis-3 16.1 15.1 25.9</td>
</tr>
<tr>
<td>b</td>
<td>SP 1200 10％</td>
<td>10 1</td>
<td>3</td>
<td>trans-3 19.0 18.0 31.5</td>
</tr>
<tr>
<td>c</td>
<td>SP 1200 10％</td>
<td>10 1</td>
<td>3</td>
<td>trans-2 9.2 8.5 12.7*3</td>
</tr>
<tr>
<td>d</td>
<td>SP 1200 5％</td>
<td>15 1</td>
<td>1.5</td>
<td>4.3 3.9 6.5</td>
</tr>
<tr>
<td>e</td>
<td>EXTT-19 15％</td>
<td>5 1</td>
<td>3</td>
<td>14.8 13.5 24.0*4</td>
</tr>
<tr>
<td>f</td>
<td>DEGS 15％</td>
<td>5 1</td>
<td>1.5</td>
<td>7.0 6.2 10.9</td>
</tr>
<tr>
<td>g</td>
<td>PEG 20 M 20％</td>
<td>5 -</td>
<td>3</td>
<td>14.5 14.4 24.5</td>
</tr>
<tr>
<td>h</td>
<td>PEG 20 M 20％</td>
<td>- -</td>
<td>3*2</td>
<td>2.5 2.5 3.5</td>
</tr>
</tbody>
</table>

*1 On Celite 545 80–100 mesh, AW DMCS glass column (3 mm ø), Column temp. 80 °C, Injector and Detector temp. 120 °C, Carrier gas: N2 50 ml/min, Shimadzu GC-6A; *2 Steel column; *3 48% Isomerization; *4 60% Isomerization.

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