Biosynthesis of Umbelliferose in *Aegopodium podagraria*¹

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**ABSTRACT**

The following reaction leading to the synthesis of the tri saccharide umbelliferose was demonstrated in an enzyme preparation from leaves of *Aegopodium podagraria* L.: sucrose + UDP-gal-¹⁴C → umbelliferose-¹⁴C + UDP. Neither galactinol nor galactose 1-phosphate could replace UDP-gal. Among 10 different sugars tested only sucrose was a suitable galactosyl acceptor.

The trisaccharide umbelliferose (O-α-D-galactopyranosyl-(1-2)-O-α-D-glucopyranosyl-(1-2)-O-β-D-fructofuranoside) was found and identified by Wickstrom and Svendsen (9). Although it is very widespread within the umbelliferae (1, 2, 7), its biosynthesis is hitherto unknown. Since raffinose, another much more widespread galactoside of sucrose, is formed by a transfer reaction in which galactinol is the galactosyl donor (4), we studied the occurrence of galactinol in umbellifera. Only traces of galactinol could be found in such leaves, which also contained very small amounts of raffinose in addition to umbelliferose. No correlation between the occurrence of umbelliferose and galactinol could be found, however (3). Also no other unusual galactoside which could serve as galactosyl donor was detected in labeling experiments similar to the studies of Senser and Kandler (6). It was, therefore, suggested that the galactosyl donor in the biosynthesis of umbelliferose may be a nucleotide-activated galactose or galactose 1-phosphate, both present in leaves of umbellifera. This paper describes an enzyme preparation from the leaves of *Aegopodium podagraria* which transfers galactose specifically from UDP-gal to position 2 of sucrose, yielding umbelliferose.

**MATERIALS AND METHODS**

The enzyme was prepared as follows. Fifteen g of fully expanded leaves of *Aegopodium podagraria* L. grown in the open air were frozen in liquid nitrogen and ground in a mortar. After mixing with the same weight of polyvinylpyrrolidone (5), the powder was extracted in the cold with 100 ml of 0.1 M tris-HCl-buffer (pH 7.0) containing 10 mM dithioerythritol. The mixture was filtered through cheesecloth, and the filtrate was centrifuged at 20,000g. The raw extract was immediately fractionated by the addition of (NH₄)₂SO₄. The fraction between 35 and 55% (NH₄)₂SO₄ was dissolved in 1 ml of 0.1 M tris-HCl-buffer, pH 7.5, containing 10 mM DTE,² applied to a Sephadex G-25 column and eluted by the same buffer. The protein peak which contained all the protein was used in the subsequent experiments as enzyme solution. No enzyme activity was found when the addition of polyvinylpyrrolidone or the gel filtration were omitted.

In all experiments, the incubation mixture contained 0.1 ml of enzyme solution (10 mg protein/ml) and 0.05 ml of each of the other substances dissolved in 0.1 M tris-HCl-buffer, pH 7.5, containing 10 mM DTE. Unlabeled substrates were added at an amount of 1 μmole per assay. If not otherwise stated, the amount of radioactivity added was as follows: 2.5 μc sucrose-¹⁴C (60 μc/μmole), 0.25 μc UDP-gal-¹⁴C (273 μc/μmole), 0.1 μc galactinol-¹⁴C (17 μc/μmole), 0.1 μc raffinose-¹⁴C (23 μc/μmole). The increase in the total amount of compounds by the addition of labeled compounds was negligible, since the specific activity was very high.

Sucrose-¹⁴C and UDP-gal-¹⁴C were purchased from American (England), labeled raffinose and umbelliferose were isolated from leaves of *Lamium maculatum* and *Aegopodium podagraria*, respectively, after the leaves were allowed to photosynthesize in CO₂ for several hours (6).

The enzyme reactions were run at 32 C and stopped with 0.8 ml of 95% ethanol. The supernatant was chromatographed on Whatman No. 1. The following solvent systems were used: I: 88% phenol-acetic acid-1 M EDTA-water (840:10:1 M, v/v); II: 1-butanol-water (solution 1)-propionic acid-water (solution 2) (1:1); solution 1: 1-butanol-water = 750:50 (v/v); solution 2: propionic acid-water = 352:448 (v/v); III: 1-butanol-pyridine-water-acetic acid (solution 3) 60:40:30:3 (v/v); IV: 1-butanol-ethylacetate-acetic acid-water = 4:3:2:5:4 (v/v).

Paper electrophoresis was carried out in 0.05 M sodium tetraborate, pH 9.8, and in 0.1 M ammonia formate, pH 3.7. The radioactive areas were located by autoradiography. The radioactivity was measured directly on paper with a methane flow counter Frieske and Hoepfner 407 A.

**RESULTS AND DISCUSSION**

To demonstrate the synthesis of umbelliferose from sucrose and various galactosyldonors, as well as the exchange reaction between umbelliferose and sucrose, the combinations of labeled and unlabeled acceptors and donors listed in Table 1 were used. Only in assays No. 2 and 3 labeled umbelliferose could be found on the one-dimensional chromatogram (solvent III). Unlike the results of similar experiments on raffinose (5) and stachyose biosynthesis (8), no exchange reaction could be observed.

To ensure that the reaction product was umbelliferose, co chromatography and co-electrophoresis with authentic umbelliferose isolated from *Carum carvi* were carried out using the four solvent systems and the two buffers mentioned above. Complete identity was achieved in all cases. When treated with e-galactosidase (pH 5.6, 12 hr), the only radioactive compound

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² Abbreviation: DTE: dithioerythritol.
formed from the reaction product was galactose-C14 when UDP-gal-C14 was used, whereas it was sucrose-C14 when sucrose-C14 was originally added to the assay. The reaction product was stable against invertase as was umbelliferose.

To demonstrate the acceptor specificity of the galactosyl transferase present in the enzyme preparation, the following possible acceptors were added together with UDP-gal-C14: α-galactose, α-glucose, melibiose, lactose, maltose, cellobiose, trehalose, raffinose, and umbelliferose. No galactose-containing new oligosaccharide was formed in any of these assays. Therefore the UDP-gal:sucrose 2-galactosyltransferase (EC 2.4. 1.?) present in the enzyme preparation shows a very pronounced specificity for sucrose as the acceptor and UDP-gal as the donor. No decrease of enzyme activity was found after 1 month of storage at −20 °C.

The enzyme preparation used was contaminated with several other enzymes. Among others it contained invertase and α-galactosidase, since sucrose and umbelliferose are split into their monomers. Fortunately, the hydrolysis of umbelliferose

Table 1. Formation of Umbelliferose from Sucrose and Various Galactosyl Donors by an Enzyme Preparation from Aegopodium podagaria

<table>
<thead>
<tr>
<th>Type of expected reaction</th>
<th>Acceptor</th>
<th>Donor</th>
<th>Umbelliferose-C14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Synthesis</td>
<td>Sucrose-C14</td>
<td>Galactinol</td>
<td>cpm</td>
</tr>
<tr>
<td>2. Synthesis</td>
<td>Sucrose-C14</td>
<td>UDP-galactose</td>
<td>8,000</td>
</tr>
<tr>
<td>3. Synthesis</td>
<td>Sucrose</td>
<td>UDP-galactose-C14</td>
<td>6,000</td>
</tr>
<tr>
<td>4. Synthesis</td>
<td>Sucrose-C14</td>
<td>Galactose-1-P</td>
<td></td>
</tr>
<tr>
<td>5. Exchange</td>
<td>Sucrose-C14</td>
<td>Umbelliferose</td>
<td></td>
</tr>
<tr>
<td>6. Exchange</td>
<td>Sucrose-C14</td>
<td>Raffinose</td>
<td></td>
</tr>
<tr>
<td>7. Control</td>
<td>Sucrose-C14</td>
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</tbody>
</table>

is much slower than its synthesis at the pH used. Figure 1 shows the influence of pH on the activity of the hydrolases and the transferase. Although the transferase activity is certainly falsified at the lower pH values by the action of α-galactosidases present in the crude enzyme preparation, the different pH optima of the hydrolases and the transferase is evident.

As shown in Figure 2, most of the UDP-gal-C14 added is converted to UDP-glu within 1 hr by UDP-gal-4-epimerase. Therefore, the rate of umbelliferose synthesis decreases very early. After 3 hr most of the radioactivity is located in glucose, fructose and sucrose arisen from UDP-glu. The umbelliferose present after 1 hr incubation contains label exclusively in the galactose moiety, while after 3 hr incubation some label (3–5%) is also present in the sucrose portion of umbelliferose. This indicates, that the preparation contains also sucrose synthetase. The purification of the transferase is under investigation.

The demonstration of a UDP-gal:sucrose 3-galactosyl transferase in leaves of Aegopodium podagaria shows, that unlike the biosynthesis of the raffinose sugars galactinol is not involved in the biosynthesis of umbelliferose. This agrees with the observation that galactinol is not significantly labeled in leaves of umbellifers during photosynthesis in 14CO2, when only umbelliferose is synthesized but only when raffinose is formed in addition (3).

**Fig. 1.** Influence of pH on several hydrolases and the UDP-gal: sucrose 2-galactosyl transferase. The assay contained in a total volume of 0.2 ml 1 mg of protein, 0.1 M phosphate buffer + 10 mM DTE and 1 umole of substrate-C14 = 0.1 μc. Incubation time 2 hr. 1: α-Galactosidase (liberation of galactose from umbelliferose); 2: α-galactosidase (liberation of galactose from raffinose); 3: invertase (liberation of fructose from sucrose); 4: UDP-gal:sucrose 2-galactosyl transferase. (Synthesis of umbelliferose-C14 from sucrose and UDP-gal-C14)

**Fig. 2.** Amount of reaction products formed from UDP-gal-C14 in relation to incubation time. 1: Umbelliferose × 10; 2: UDP-galactose 3: UDP-glucose; 4: sucrose, glucose, fructose. The assay contained in a total volume of 0.2 ml 0.1 M tris-HCl buffer + 10 mM DTE, pH 7.5, 1 mg of protein, 1 μmole of UDP-gal-C14 = 1 μc, 1 μmole of sucrose.

**LITERATURE CITED**