

PROOF FOR THE ORIGIN OF THE BRANCH HYDROXYMETHYL CARBON OF D-APIOSE FROM CARBON 3 OF D-GLUCURONIC ACID

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1. Introduction

The results of an earlier experiment in which D-3, 4-¹⁴C₂-glucose was fed to shoots of young parsley plants proved that C-3' of apiose (3-C-hydroxymethyl-D-erythro-furanose) must originate from either C-3 or C-4 of glucose [1]. Beck and Kandler [2] determined the distribution of radioactivity on the carbon atoms of apiose, xylose and glucose isolated from *Lemna* plants which had been grown in the presence of D-1-¹⁴C-glucose and D-2-¹⁴C-glucose. They found very good agreement between the radioactivity of C-3 of apiose and C-4 of glucose. According to these results and in analogy with the biosynthesis of L-streptose from D-1,3-¹⁴C₂-glucose [3], it was assumed that the rearrangement leading to apiose occurs with expulsion of C-3 of D-glucuronic acid [1, 4]. With the availability of an enzyme catalyzing the conversion of uridine diphospho-D-glucuronic acid (UDP-GlcUA) to uridine diphospho-D-apiose (UDP-Api) [5, 6] it was now possible to prove unequivocally the origin of C-3' of apiose with UDP-3-¹⁴C-GlcUA as substrate.

2. Materials and methods

2.1. Materials

D-3-¹⁴C-glucose (10 μ Ci/ μ mole) was purchased from New England Nuclear (Boston, Mass., USA). The supplied solution was evaporated to dryness *in vacuo* and the residue was dissolved in an appropriate volume of distilled water.

2.2. Separation and analysis

Schleicher-Schüll paper No. 2043 b was used for chromatography. UDP-GlcUA was separated from incubation mixtures by chromatography with 95% ethanol–1 M ammonium acetate, pH 7.5 (5:2, v/v) [8]. Apiin was separated from incubation mixtures by chromatography with 15% aqueous acetic acid and paper which had been previously washed with 0.01 M EDTA, pH 7.0, and then with distilled water.

High-voltage paper electrophoresis used for the purification of UDP-GlcUA was carried out at 2000–2500 V for 50–60 min with a Pherograph Frankfurt (Hormuth u. Vetter, Wiesloch, Germany) on 30 × 40 cm sheets of Macherey-Nagel paper No. 214 wetted with 0.15 M triethylammonium acetate buffer, pH 4.4 [9]. The same buffer was used in the electrolyte vessels.

Radioactivity measurements were made with a Packard Tri-Carb Model 3375 liquid scintillation spectrometer. The samples were counted after dissolving in 20 ml of a solution consisting of 0.5% 2, 5-diphenyl-oxazole (PPO) and 10% naphthalene in dioxane. Corrections for counting efficiency were made through the use of standard ¹⁴C-*n*-hexadecane as an internal standard.

2.3. Preparation of enzymes

UDP-apiose synthetase was prepared from extracts of cell-suspension cultures of parsley and purified up to the Sephadex G-200 step [6].

UDP-apiose:7-glucosylapigenin apiosyl transferase

Table 1
Periodate oxidation of apiin obtained from UDP-3-¹⁴C-glucuronic acid by the combined action of UDP-apiose synthetase and UDP-apiose transferase.

Compound	Radioactivity (dpm)	Percent of apiin
Apiin		
1st crystallization	10,120	
2nd crystallization	10,620	
3rd crystallization	8,976	
Formaldehyde-dimedone from 3rd apiin crystallization		
1st crystallization	8,702*	96
2nd crystallization	9,024*	101

* Corrected for dilution with carrier and to 100% yield. Actual yield of HCHO-dimedone derivative was 80% of theory.

was prepared from cell cultures of parsley and purified up to the Sephadex G-100 step [10].

2.4. Preparation of UDP-D-3-¹⁴C-GlcUA

UDP-D-3-¹⁴C-GlcUA was produced enzymatically from D-3-¹⁴C-glucose (5 μ Ci) through the combined action of hexokinase, phosphoglucosyltransferase, UDPG pyrophosphorylase, and UDPG dehydrogenase as described earlier [7]. The yield of UDP-D-3-¹⁴C-GlcUA, as calculated from NAD⁺ reduction, was 62.5% after 135 min of incubation. The incubation mixture was treated with alkaline phosphatase and the UDP-D-3-¹⁴C-GlcUA purified alternately by paper chromatography and paper electrophoresis [7].

3. Results and discussion

UDP-3-¹⁴C-GlcUA was incubated with the enzyme from cell-suspension cultures of parsley which had been purified up to the Sephadex G-200 step [6]. In order to obtain an apiose derivative which was reasonably stable and which could be produced without passing through the aldehyde form, apiose from UDP-Apio was then transferred to 7-O-(β -glucosyl)-apigenin with a transferase preparation from parsley cell cultures [7, 10]. The product of this reaction, apiin (7-O-[β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-glucosyl]-5, 7, 4'-trihydroxy-flavone) was diluted with carrier apiin and recrystallized from ethanol to constant specific activity. The labeled apiin was then oxidized with periodate in

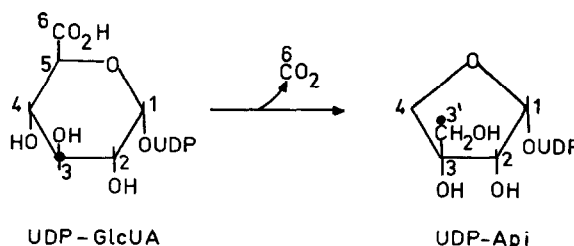


Fig. 1.

sodium bicarbonate solution [1] and the formaldehyde originating from C-3' of apiose trapped as dimedone derivative. The table shows the ¹⁴C activities in apiin and the formaldehyde-dimedone derivative. All radioactivity is located, within the limits of experimental error, at C-3' of apiose. This proves without doubt the origin of the branch hydroxymethyl carbon of D-apiose from C-3 of D-glucuronic acid. This result further underlines the similarity in the rearrangement of the sugar chain leading to L-streptose [3] and D-apiose.

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