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-$^{14}C_2$-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-^{14}C-glucose and D-2-^{14}C-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-$^{14}C_2$-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-^{14}C-GlcUA as substrate.

2. Materials and methods

2.1. *Materials*

D-3-^{14}C-glucose (10 μCi/μmole) 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-diphenyloxazole (PPO) and 10% naphthalene in dioxane. Corrections for counting efficiency were made through the use of standard ^{14}C-*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-^{14}C-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-^{14}C-GlcUA*

UDP-D-3-^{14}C-GlcUA was produced enzymatically from D-3-^{14}C-glucose (5 μCi) through the combined action of hexokinase, phosphoglucomutase, UDPG pyrophosphorylase, and UDPG dehydrogenase as described earlier [7]. The yield of UDP-D-3-^{14}C-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-^{14}C-GlcUA purified alternately by paper chromatography and paper electrophoresis [7].

3. Results and discussion

UDP-3-^{14}C-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 aldehydo form, apiose from UPD-Api 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→2)-β-D-glucosyl]-5, 7, 4′-trihydroxyflavone) was diluted with carrier apiin and recrystallized from ethanol to constant specific activity. The labeled apiin was then oxidized with periodate in

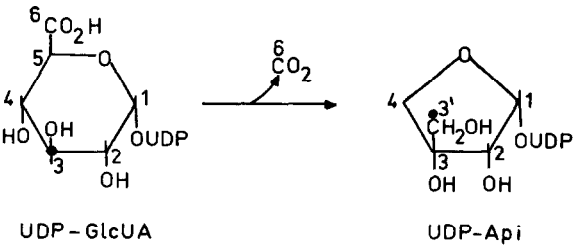

Fig. 1.

sodium bicarbonate solution [1] and the formaldehyde originating from C-3′ of apiose trapped as dimedone derivative. The table shows the ^{14}C 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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References

[1] H. Grisebach and U. Döbereiner, Biochem. Biophys. Res. Commun. 17 (1964) 737; Z. Naturforsch. 21 b (1966) 429.
[2] E. Beck and O. Kandler, Z. Pflanzenphysiol. 55 (1966) 71.
[3] D.J. Candy and J. Baddiley, Biochem. J. 96 (1965) 526.
[4] H. Grisebach and H. Sandermann, Biochem. Z. 346 (1966) 322.
[5] E. Wellmann and H. Grisebach, Biochim. Biophys. Acta 235 (1971) 389.
[6] D. Baron, E. Wellmann and H. Grisebach, Biochim. Biophys. Acta 258 (1972) 310.
[7] W.J. Kelleher and H. Grisebach, European J. Biochem. 23 (1971) 136.
[8] K.C. Tovey and R.M. Roberts, J. Chromatogr. 47 (1970) 287.
[9] J.A. Thomas, K.K. Schlender and J. Larner, Anal. Biochem. 25 (1968) 486.
[10] H. Grisebach and R. Ortmann, in: Methods of Enzymology, eds. S.P. Colowick and N.O. Kaplan (Academic Press) in press.