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SEDOHEPTULOSE IN PHOTOSYNTHESIS BY PLANTS

A. A. Benson, J. A. Bassham, and M. Calvin

May 1, 1951

Berkeley, California

SEDOHEPTULOSE IN PHOTOSYNTHESIS BY PLANTS\*

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Radiation Laboratory and Department of Chemistry

University of California, Berkeley

May 1, 1951

Although its function has not been ascertained, the general occurrence of sedoheptulose<sup>1</sup>, D-altrioheptulose, in the succulent plants is well established. This sugar has not been identified in the majority of the members of the plant kingdom, but it now appears possible that its phosphate esters may perform a vital function during photosynthesis.

We have isolated labeled sedoheptulose monophosphate in  $C^{14}O_2$  photosynthesis products of all the plants thus far studied in this laboratory (Chlorella, Scenedesmus, Rhodospirillum rubrum, and the leaves of barley seedlings, soy bean, alfalfa, sugar beet, spinach and geranium). It is invariably found as monophosphate esters. At least two such esters have been observed in radiograms of  $C^{14}$ -labeled Scenedesmus. The major one is associated with fructose monophosphate while the minor one is inseparable, as yet, from glucose monophosphate. Sedoheptulose may be liberated enzymatically from its phosphates during the killing of the plant, but it has not been observed to accumulate in amounts exceeding the steady state concentrations of these phosphates.

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This suggests its participation only as a phosphate in most plants. These sedoheptulose phosphates are formed prior to hexose phosphates in the cases examined kinetically in this laboratory. In a typical experiment, one-second photosynthesis in  $C^{14}O_2$  by barley seedling leaves, the distribution of radioactivity among the neutral compounds obtained upon phosphatase hydrolysis of the mixed phosphates was as follows: 43% in fructose, 47% sedoheptulose and 7% in glucose.

Sedoheptulose, isolated chromatographically<sup>2</sup> from phosphatase ("Polidase") hydrolysates of similarly separated phosphate esters<sup>2</sup>, was identified by the following tests. (1) two-dimensional paper co-chromatography with authentic sedoheptulose<sup>3</sup> showed identical positions of the sugar and the radioactivity. The position of the authentic specimens was determined by resorcinol spray test. (2) The radioactive sugar in tracer concentrations is readily converted to sedoheptulosan by five-minute heating in 1N hydrochloric acid. It was identified by co-chromatography with sedoheptulosan prepared similarly from an authentic specimen. (3) The equilibrium constant of the dehydration of a radioactive compound was found to be 4.0 as reported by LaForge and Hudson<sup>1</sup> for sedoheptulose. (4) Catalytic hydrogenation gave D- $\beta$ -mannoheptitol, which was identified by co-chromatography with an authentic specimen prepared from sedoheptulose. (5) Periodate oxidation of both the hexose and the heptitol gave the expected amounts of products. The sedoheptulose obtained from five minutes  $C^{14}O_2$  photosynthesis by soy bean leaves gave 14.4% of formaldehyde activity, 28% glycolic acid activity

and 55% of formate activity. The heptitol obtained from this compound had a formate/formaldehyde ratio of 3.1 compared to an expected 2.5 for uniform labeling.

The examination of the kinetics of formation of the phosphate esters involved in  $C^{14}O_2$  fixation<sup>4</sup> and a detailed description of the identification will be published.

This early synthesis of sedoheptulose in  $CO_2$  fixation and its stereochemical deviation from that of glucose strongly suggests its participation in a  $C_2$  regenerative system for the primary  $CO_2$ -acceptor rather than as a hexose precursor. The predominant role of malic acid in "succulent metabolism" may well be related to the accumulation of sedoheptulose in these plants.

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REFERENCES

- (1) F. B. LaForge and C. S. Hudson, J. Biol. Chem., 30, 61 (1917).
- (2) Benson, Bassham, Calvin, Goodale, Haas, Stepka, J. Am. Chem. Soc., 72, 1710 (1950).
- (3) A sample was kindly supplied by Mr. E. W. Putman of the Division of Plant Nutrition of this University.
- (4) Benson, Kawaguchi and Calvin, to be published.