Title

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Publication Date

1955-04-22

UCRL 2971

UNCLASSIFIED

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

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UCRL-2971
Unclassified Health and Biology

UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

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Printed for the U. S. Atomic Energy Commission

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ABSTRACT

The biosynthesis of sedoheptulose in the Sedum plant is studied to develop procedures for synthesizing C^{14}-labeled sedoheptulose in optimal yield. Preliminary investigations showed that very small amounts of C^{14}-labeled sedoheptulose were formed during the first 24 hours of photosynthesis in $C^{14}O_2$.

The formation of C^{14}-labeled sedoheptulose as well as its relationship to the other C^{14}-labeled compounds--especially sucrose, glucose, and fructose--was studied. The rate of accumulation of free sedoheptulose in Sedum spectabile varied considerably with the age of the plants, the conditions under which the plants were kept before the experiments (atmospheric conditions, light and dark treatments), and the medium (water or nutrient solution) in which the plants were kept during the photosynthesis.

Free sedoheptulose accumulates much more slowly than do sucrose, glucose and fructose, and, likewise, is only slowly depleted. Starved plants deprived of their reservoir of free hexoses still contained remarkable amounts of free sedoheptulose. In $C^{14}O_2$ they were always found to accumulate very little free radioactive sedoheptulose until the reservoirs of hexoses were restored. It is concluded that the unique sedoheptulose accumulation in Sedum is largely due to the relative inactivity of the required kinase.

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INTRODUCTION

Preliminary Investigations

Bennett-Clark[1] reported that in certain Sedum species the amount of free sedoheptulose decreased during the night and rose again during the day in a manner complementary to that of malic acid. This does not seem to have been observed by other investigators.[2,3] Vickery[4] reported that sedoheptulose was formed very slowly in Bryophyllum. Recently, Tolbert and Zill[5] prepared sedoheptulose-C^{14} using S. spectabile and observed labeling of sucrose, glucose, fructose, and sedoheptulose after 26 hours of photosynthesis in $C^{14}O_2$. Their yield of sedoheptulose-C^{14} was not optimal, and it was clear that an understanding of the formation and metabolism of sedoheptulose is required for development of improved phytosynthetic preparation of the labeled compound.

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* The work described in this paper was sponsored by the U. S. Atomic Energy Commission, the Rockefeller Foundation, and the Norges Almenligvitenskapelige Forskningsråd.

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MATERIALS AND METHODS

We reinvestigated the rate of sedoheptulose synthesis and depletion in some preliminary experiments. These required plants such as Sedum confusum, Sedum dendroideum (praealtum), and Sedum spectabile L. with normally high sedoheptulose content which would remain turgid at high light intensity for extended periods. Test plants (without roots) were kept in small beakers in water or nutrient solutions in a growth chamber (an empty desiccator) closed with a tight-fitting glass cover in which there were an inlet and an outlet for the gas. The sedoheptulose content was observed as a function of light and of atmospheric and temperature variations. The light intensity was 375 foot-candles from blue and pink fluorescent lamps. The flowing atmospheres were (1) air containing 4% CO_2, (2) CO_2-free air, and (3) nitrogen.

To assay approximate contents of free sedoheptulose, sucrose, and fructose in the plants before, during, and after the experiments, a certain amount of expressed sap (10 to 50 µl) or a corresponding amount of alcoholic extract of the plants* was chromatographed undimensionally in phenol for 24 hours and then tested with the orcinol-TCA spray.[6] From the relative size and intensity of the spots it was possible to get a rough (but, for the purpose, satisfactory) picture of the variations in the content of the free sugars in the plants. In testing for sedoheptulose alone, the juice was expressed on a filter paper and sprayed directly.

The main results of the preliminary experiments can be summarized as follows:

(a) Free sedoheptulose in the Sedum plants is very slowly metabolized. It required from five hours to several days to obtain clear changes in its concentration in the plant juice.

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* Because of the great content of slimy substances in the juice, the leaves of Sedum spectabile could not be pressed out directly. The plant tissues were therefore triturated in a mortar with equal parts of 95% alcohol and centrifuged, and the supernatant liquid was used.

(b) In the 4% CO_2-in-air experiment small changes up and down owing to assimilation and respiration were noticed.

(c) In CO_2-free air the amount of the free sugar gradually decreased during illumination. In this case the main part of the sugar disappeared from the juice in the course of 2 to 5 days in the Sedum confusum and Sedum spectabile. In the more thick-leaved Sedum dendroideum (praealtum) it took between 2 and 3 weeks to obtain an appreciable decay. Restoration of the depleted sedoheptulose reservoir required several days, even if the plants were kept outdoors under normal conditions.

(d) The nitrogen atmosphere prevented metabolism of sedoheptulose and was generally injurious.

(e) In Sedum confusum the sedoheptulose content differed greatly from leaf to leaf. The leaves wilted much more easily than those of Sedum spectabile.

(f) It was noticed that certain crassulacean plants, among them Sedum confusum, gradually lose their high sedoheptulose content when kept in diffused light in a greenhouse or in a laboratory for some weeks.

(g) Free fructose and sucrose in the Sedums behave normally as they increase during a light period in air (4% CO_2 in air) and disappear when the plant is kept in the dark for some hours.

(h) Of the plants tested, Sedum spectabile was found to be most suitable for preparation of sedoheptulose-C^{14} because of the following characteristics: The plant has a relatively large amount of free sedoheptulose which varies little from leaf to leaf. The leaves are thin as compared with most other Sedum species, and it is therefore relatively easy to deplete the reservoirs of free sugars before starting certain experiments. The plant can tolerate $25^{\circ}-30^{\circ}$ C for several days without wilting. It is readily propagated from buds of older plants to give small plants suitable for long-time photosynthetic experiments in $C^{14}O_2$.

Preparation of the test plants

Mature S. spectabile plants were cut and allowed to lie 1 to 2 weeks while small buds developed at the leaf corners. These rooted in soil and were grown 6 to 8 weeks before use. During the long-time experiments the plants were kept in small beakers or specially made containers (U-tubes with 2 or 3 arms) which were covered with aluminum foil to protect the roots from light, and contained either nutrient solution or distilled water.[7]

Equipment

Figure 1 shows diagrammatically the equipment used for the performance of the actual biosynthesis and for the recording of $C^{14}O_2$ content in the circulating system. Total volumes were 250 cc and 550 cc, including a 100-cc and a 400-cc illumination chamber, respectively.

The $C^{14}O_2$ was admitted into the growth chamber from the U-tube (Fig. 1, No. 4). The radioactivity in the circulating atmosphere was detected by an ionization chamber (9), which was warmed by a heating tape (10) to prevent moisture condensation. To insure a gas-tight system a slight vacuum (10 cm Hg) was maintained.

Light sources were two 150-watt reflector-floodlights (15) 20 cm away from the growth chamber, giving about 1000 foot-candles near the test plant. Water-cooled infrared filter glass was used to absorb excess heat. The light was controlled by the interval timer (17).

Analysis of the test plants

Fresh plant tissue was killed in a small mortar with 2 to 10 parts alcohol of varying concentration. The triturates were centrifuged and the supernatant liquid either was used directly for chromatography after being heated for 3 to 5 minutes in a boiling water bath, or was concentrated to a small volume before chromatography. The extracts were chromatographed on Whatman No. 1 paper in phenol-water (72:28) for 48 hours and then in butanol-propionic acid solvent[8] for 40 hours, and C^{14}-labeled compounds were defined by radiograms and measured by direct counting with a large thin-window Geiger-Mueller counter.

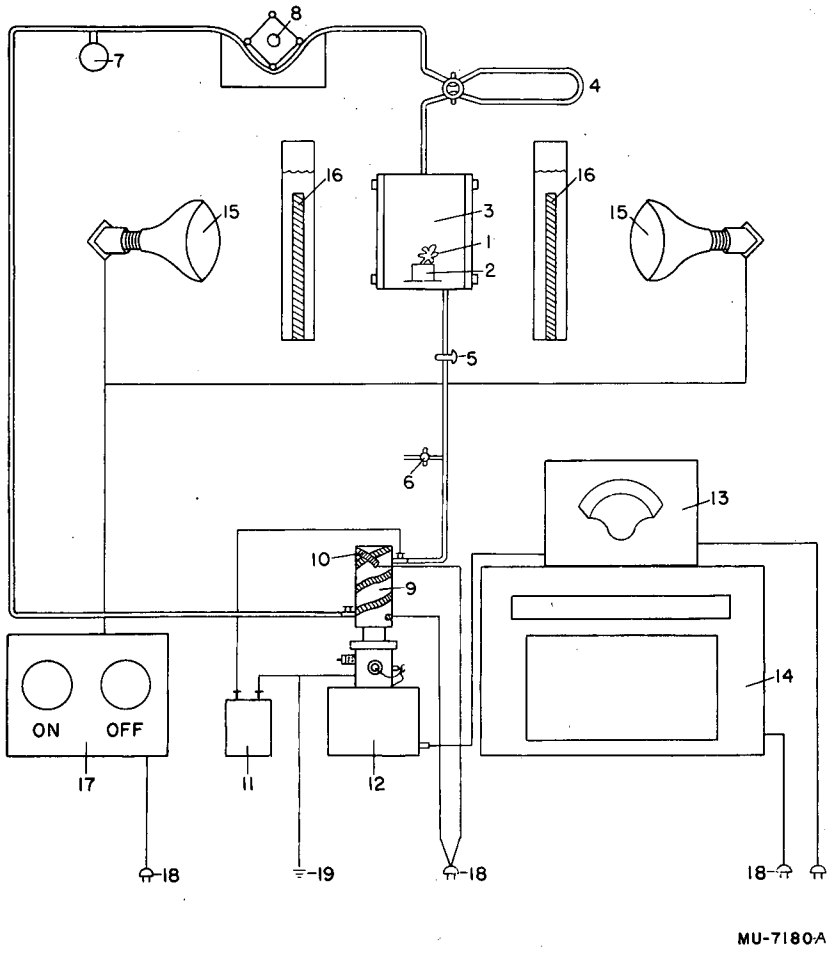

Fig. 1. Experimental Arrangement for Sedum photosynthesis: (1) Test plant; (2) beaker with water or nutrient solution; (3) growth chamber; (4) $C^{14}O_2$ container with bypass stopcock; (5) stopcock; (6) stopcock for evacuation of system; (7) vacuum gauge; (8) rubber-tubing pump; (9) ionization chamber; (10) heating tape; (11) battery, 90v; (12) vibrating-reed electrometer; (13) amplifier; (14) recorder; (15) floodlight (150w); (16) water-cooled infrared filter; (17) light-dark interval timer; (18) 110 v; (19) ground.

Phytosynthesis of Sedoheptulose-C^{14}

Experiment No. 1, two hours' photosynthesis

A young unstarved plant was kept with roots in water in a 100-cc growth chamber during the experiment and illuminated by two reflector flood lights (150 watts each).

A 2-g plant was taken directly from the greenhouse (1:00 p. m.) at the start of the experiment. About 70 μc $C^{14}O_2$ (0.15 mmole) was introduced into the system at the beginning of the experiment. The plant was killed by grinding in a mortar with four parts 95% alcohol. The mixture was centrifuged, and the supernatant extract was evaporated to 1.0 ml by a nitrogen stream over a water bath and then chromatographed. The activity in the sucrose, glucose, fructose, and sedoheptulose spots corresponded to respectively 68.0, 68.0, 58.0 and 5.8 counts per minute per mg fresh plant. The total activity in all the other visible spots on the chromatogram (largely malic acid) was 152 counts per minute. This means that about 3% of the extracted activity was found in the sedoheptulose.

Experiment No. 2 - Twenty-four hours' photosynthesis

A young plant, grown outside, was starved and kept in water during the experiment. For the actual experiment the two upper leaves plus about one inch of the stem were used. The plant used for the experiment was about 5 cm high. Before use it was starved by keeping it (with the root in water) in a light chamber in CO_2-free air in the light for about eight days and thereafter in dark for 12 hours. The two leaves plus the part of them used for the experiment amounted to 0.6 g. The amount of $C^{14}O_2$ and the experimental procedure were the same as in Experiment No. 1. The result is shown in Fig. 2. The activities in the sucrose, glucose, fructose, and sedoheptulose spots were 190, 200, 240, and 12 counts per minute per mg tissue. Total activity in the other visible spots on the chromatogram was 84 counts per minute per mg tissue. That means that 1.7% of the fixed activity was found

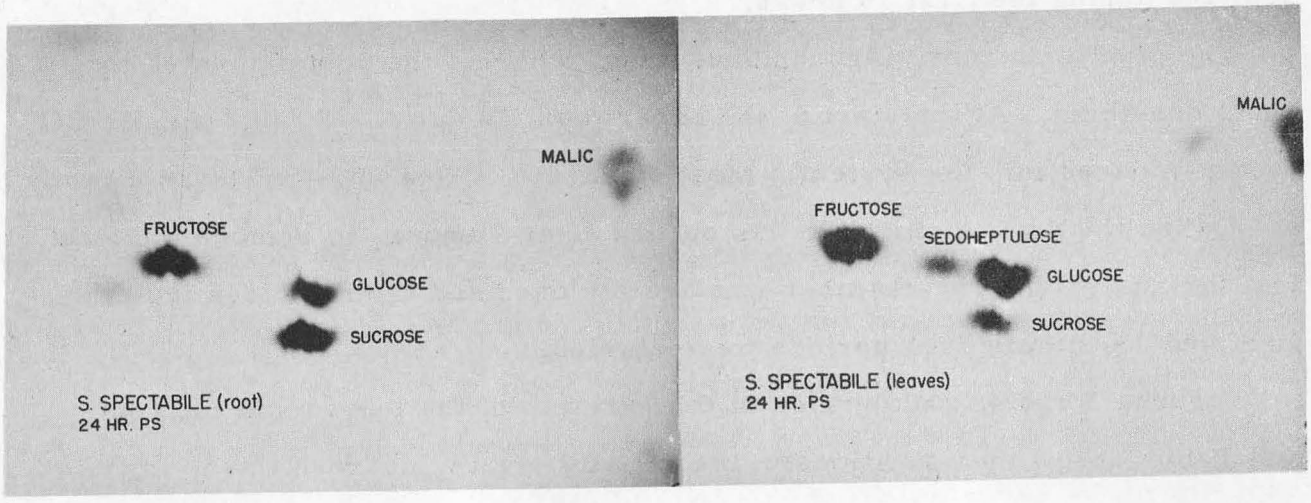

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Fig. 2. Soluble products of 24-hour photosynthesis by young S. spectabile plant.

Leaves from Experiment No. 2; root from an independent experiment.

in the sedoheptulose.

The two experiments show that in young plants free sucrose, glucose, and fructose are accumulated rapidly, while the sedoheptulose accumulates very slowly and in poor yield during the first 24 hours of photosynthesis.

Experiment No. 3 - Thirty-eight hours' photosynthesis plus 184 hours of alternating 5-minute light and 15-minute dark periods

A young unstarved plant with root was kept in water during the experiment. The system was closed during the experiment except when samples were taken out for analysis after 38, 94, 141, and 208 hours after the start of the experiment. The radioactivity in the system was recorded and found essentially depleted during the first 24 hours.

The plant with root weighed about 1.0 g, whereof the root amounted to about one-third. At the start of the experiment 145 μc $C^{14}O_2$ (0.3 mmole CO_2) was introduced into the system. Most of the $C^{14}O_2$ (ca 90%–95%) was already used when the growth chamber was opened after 38 hours to remove the first leaf for analysis. The chamber was again closed and alternate 5-minute light and 15-minute dark periods were started.

Figures 3 and 4, radiograms of the extracts of the leaves and the root, and Table I show the variations in the activity per mg of fresh tissue in the sucrose, glucose, fructose, and sedoheptulose spots during this experiment.

During the last two days of the experiment the leaves of the plant (the lower first) turned yellow, but almost without wilting.

The experiment shows a relationship between the biosynthesis of sucrose, glucose, and fructose on one hand and sedoheptulose on the other -- in such a way that the amount of free heptulose in the plant increases at the same time as the amounts of sucrose and of the free hexoses go down. The transformation from hexoses to free heptulose is a very slow process occurring during the plant's photosynthesis. There is a clear difference in the distribution of the sugars in the leaves and in the root. Sedoheptulose and fructose predominate in the leaves at the end of the experiment, while sucrose and sedoheptulose accumulate in the root.

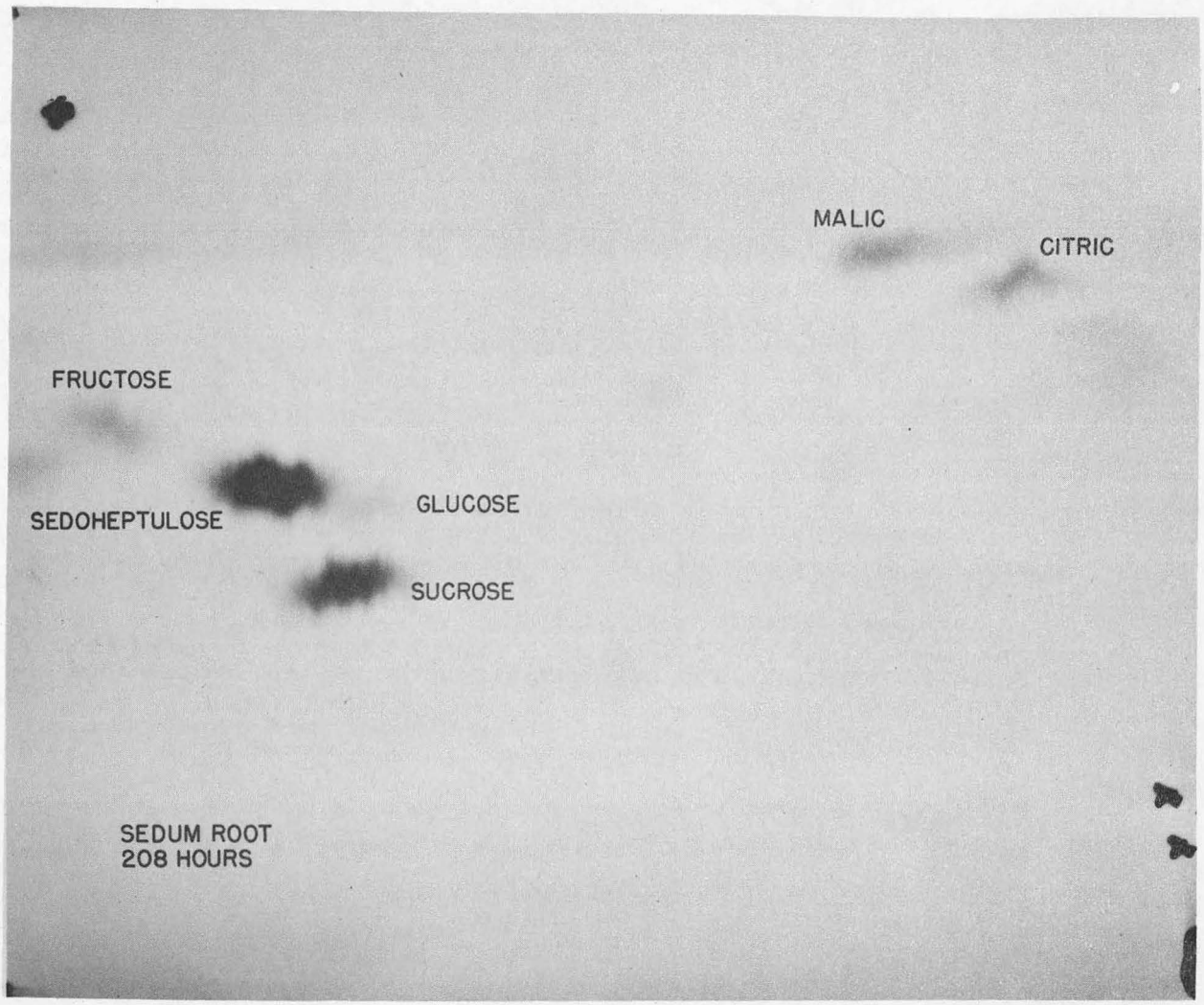

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Fig. 3. S. spectabile leaves; soluble products after 208 hours.

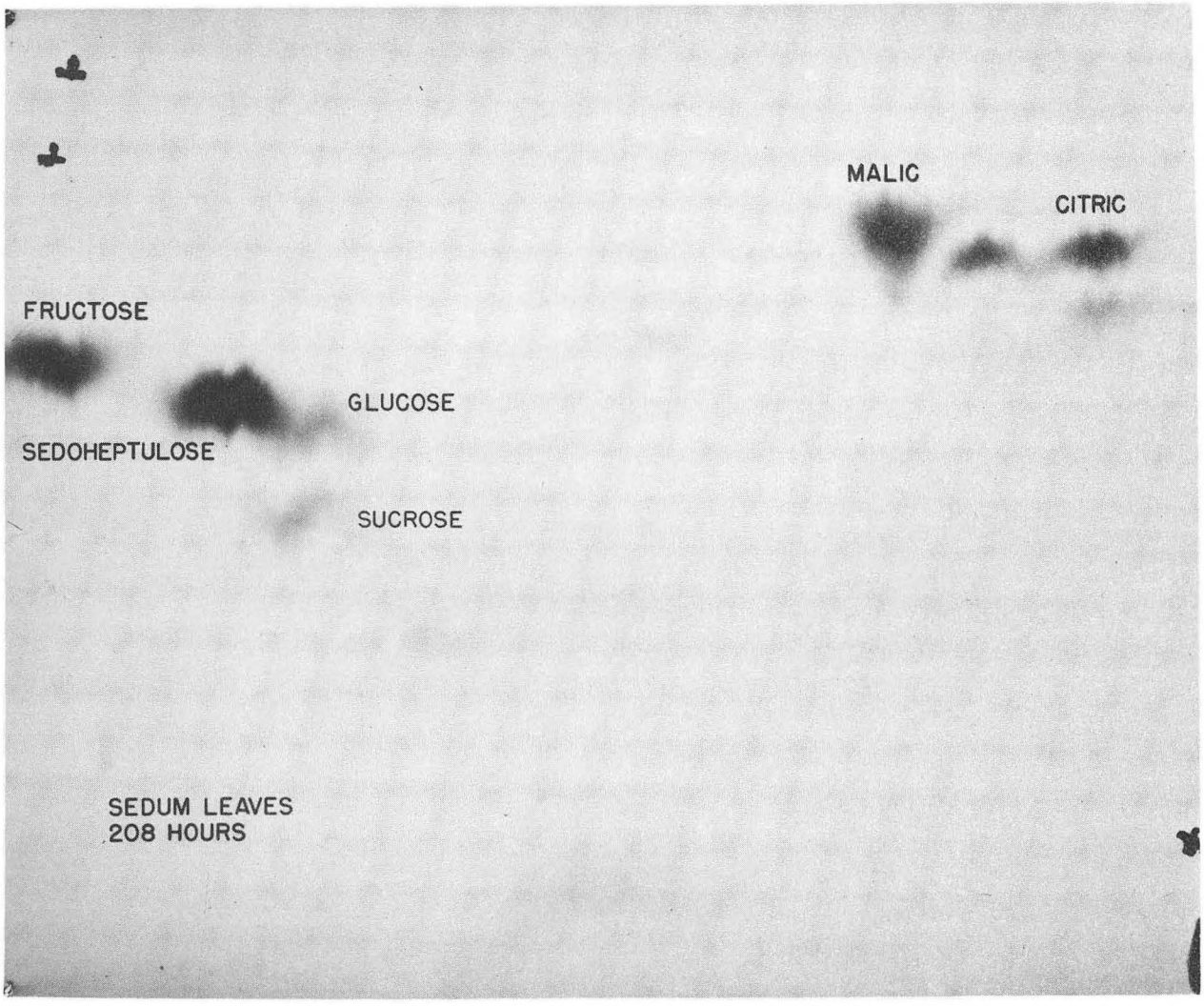

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Fig. 4. S. spectabile root; soluble products after 208 hours.

Table I

Relative C^{14} Content in Free Sugars of S. spectabile

	Leaves				Root
Sugar	Counts[a] per min after 38 hours	Counts per min after 94 hours	Counts per min after 141 hours	Counts per min after 208 hours	Counts per min after 208 hours
Sucrose	425 (39.5%)	285 (31%)	165 (20%)	73 (9%)	188 (29%)
Glucose	260 (24%)	180 (20%)	140 (17%)	88 (11%)	61 (9%)
Fructose	356 (33%)	292 (32%)	268 (32%)	239 (30%)	47 (7%)
Sedoheptulose	38 (3.5%)	156 (17%)	256 (31%)	400 (50%)	360 (55%)
Total soluble	7500	5670	4300	3916	3990

(a) Counts/min mg fresh plant tissue measured directly on two-dimensional chromatograms.

Experiment No. 4 - Twenty-six hours' photosynthesis

Young and old plants, starved and unstarved, with and without roots, in water and in nutrient solution, were kept in the same growth chamber during the experiment.

Figure 5 shows diagrammatically the setup used for the experiment. Plants 1, 3, 4, and 6 were raised in the greenhouse from buds. Before the experiment, Plants 3 and 6 were starved in CO_2-free air in water at 23° C in the following way: (a) Light 12 hours; (b) dark 24 hours; (c) light 12 hours; (d) dark 12 hours. Plants 1 and 4 were taken from the green house just before the experiment. Numbers 2 and 5 were opposite leaves from the same adult plant (15 cm high) grown outside and harvested in the morning after a previous hot, sunny day. In 6b the lower part of the root was lacking. About one mc $C^{14}O_2$ (0.5 mmole CO_2) was introduced into the system at the start of the experiment.

After 26 hours of photosynthesis the test plants were ground (roots extracted separately) with five parts of 80% alcohol. Table II shows the total soluble activity fixed per mg of fresh tissue, and gives the total soluble activity and the activity found in the individual sugar spots as calculated per mg fresh plant tissue. The following conclusions can be drawn from the experiment:

(a) Prèstarved plants fixed somewhat more C^{14} than the unstarved plants (i. e. 8, 000/4, 000 and 19, 000/15, 000).

(b) Plants that were kept in nutrient solution fixed more $C^{14}O_2$ than the plants kept in water.

(c) The stem and leaves of plants without roots showed a relatively larger amount of sedoheptulose than the corresponding parts of the plants which also had roots. This indicates that the sedoheptulose formed in the leaves is translocated to the root where it takes its place as a storage product.

(d) Unstarved plants showed a relatively larger amount of sedoheptulose

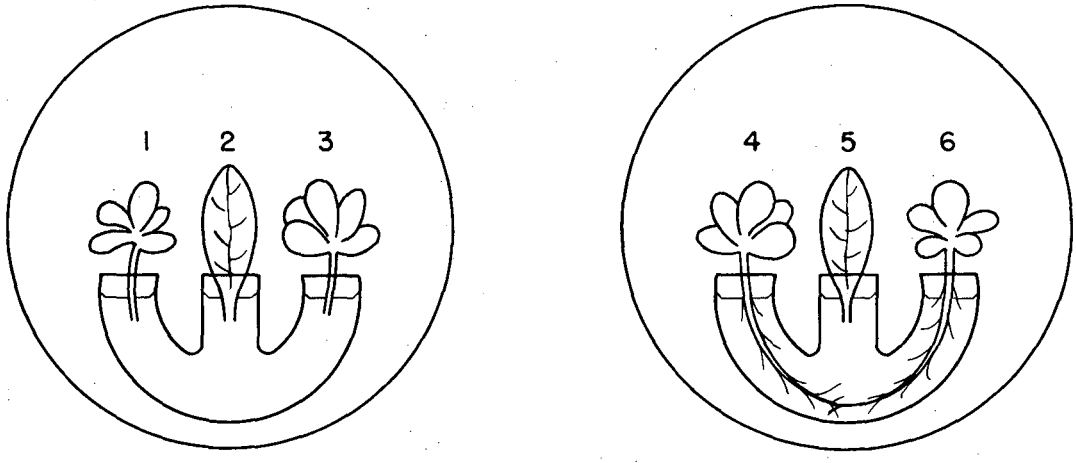

Fig. 5. Arrangement of Sedum spectabile specimens in illumination chamber (Experiment 4).

Table II

Relative C^{14} Content* of Free Sugars Formed During 26 Hours' Photosynthesis by Samples of S. spectabile

	In water			In nutrient solution				
Age	Young	Mature	Young	Young		Mature	Young	
Condition	Normal	Normal	Starved	Normal		Normal	Starved	
Part	leaves	leaf	leaves	leaves	root	leaf	leaves	root
Soluble extract	3,870	2,600	7,900	14,500	350	6,200	16,000	2,810
Sucrose	345	452	950	1,150	60	1,740	1,460	640
Glucose	632	448	960	1,500	11	1,220	1,250	410
Fructose	705	420	1,210	1,300	14	2,400	1,760	580
Sedoheptulose	164	115	152	810	7	1,540	305	15

* Counts per minute per mg fresh plant tissue measured on two-dimensional paper chromatograms.

than the starved plants. In these plants the main part of the activity was formed in the sucrose, glucose, and fructose. This seems to indicate that the sedoheptulose-7-phosphate first formed in the starved plant is rapidly used in formation of CO_2 acceptors for photosynthesis and that the reservoirs of the free hexoses' products of photosynthesis are filled before the plant accumulates free sedoheptulose.

(e) Adult plants accumulate free radioactive sedoheptulose more rapidly than do younger plants. For the biosynthesis of C^{14}-labeled sedoheptulose, therefore, adult unstarved plants should be used for periods of five days or more.

Discussion

Free sedoheptulose is almost certainly derived from its phosphate, which is intimately involved in the transketolase-catalyzed equilibration of the ketose monophosphates involved in plant metabolism (Fig. 6). The concentration of such a sugar in a plant is a result of its steady-state rates of synthesis and conversion. There are two possible mechanisms for its anomalous accumulation in the succulents. They may possess a particularly active and specific phosphatase for sedoheptulose-7-P--but, since the formation of free sedoheptulose is slow, this is unlikely. The free hexoses would thus arise mainly from partial sucrose hydrolysis rather than by phosphatase action on their phosphates. Alternatively, the slow depletion of the sedoheptulose reservoir compared to the quite rapid decreases in hexose concentrations indicates that sedoheptulokinase is not comparable in activity to the hexokinases. The specific sedoheptulokinase observed in a variety of leaves by Tolbert and Zill[5] may be very slow in Sedum. The sluggish metabolism of free sedoheptulose in these plants, then, may be due to an enzymatic deficiency. The outstanding ability of the Crassulacean plants to withstand long dry periods can be due to the presence of such a large, slowly metabolized reserve energy source.

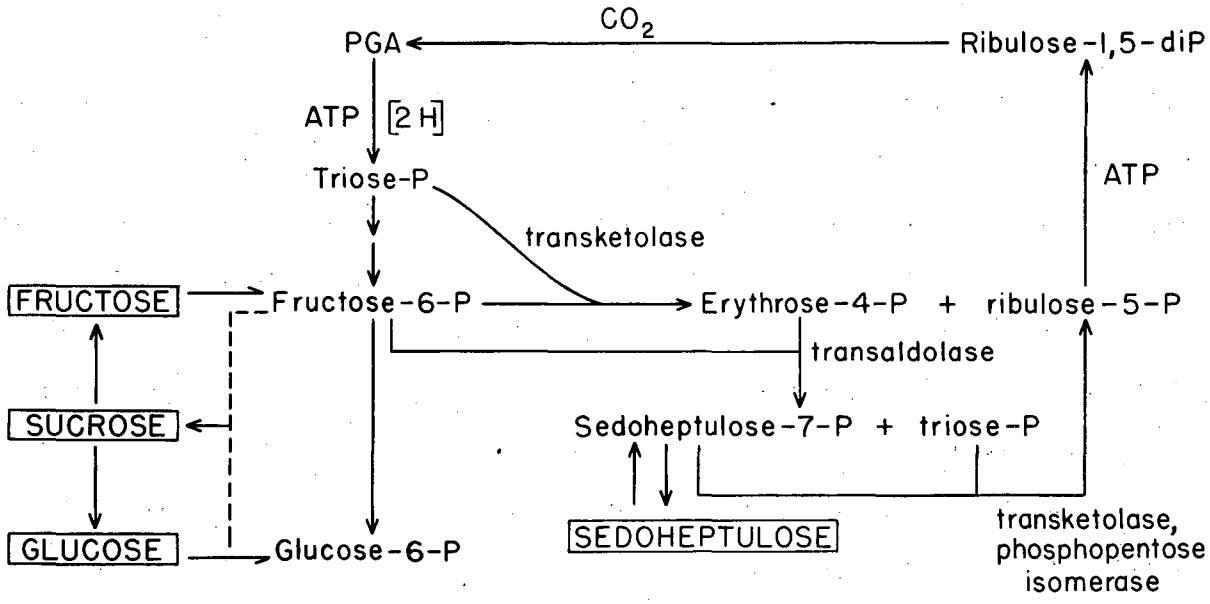

Fig. 6. Mechanism of sugar accumulation in Sedum.

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