LARGE-SCALE PHOTOSYNTHETIC PRODUCTION OF CARBON-13 LABELED SUGARS: THE TOBACCO LEAF SYSTEM

V. H. Kollman, J. L. Hanners, J. Y. Hutson, T. W. Whaley,

D. G. Ott, and C. T. Gregg

Biomedical Research Group, Los Alamos Scientific Laboratory
University of California, Los Alamos, New Mexico 87544

Received November 7,1972;revised December 13,1972

SUMMARY: A system is described for the production of carbon-13 uniformly labeled starch, glucose, fructose, and sucrose from kilogram quantities of tobacco leaves. Nearly 60% of the CO_2 administered (at 80 atom % carbon-13 or above) was found in these four compounds; the loss of enrichment between carbon-13 administered and isolated sugars was 3% or less. About 25 g ^{13}C-glucose can be produced in a 40-hr incubation from 1.2 kg of leaves. Label uniformity in the products was established by ^{13}C-nmr spectroscopy.

INTRODUCTION

For most applications the highly enriched stable isotopes produced in large quantity at the Los Alamos Scientific Laboratory (1) must be incorporated into more complex molecules either by organic or biosynthesis. Here we report the large-scale biosynthesis of uniformly carbon-13 labeled sugars from highly enriched $^{13}CO_2$. The tobacco leaf system produces primarily starch with lesser amounts of free glucose, sucrose, and fructose. Subsequent papers will cover large-scale photosynthesis with other systems.

METHODS AND MATERIALS

Plant Growth:--Tobacco plants (*Nicotiana tobacum* L., variety: Coker 319) were grown hydroponically in the laboratory from seed kindly given us by

---

*A portion of the highly enriched carbon-13 produced at the Los Alamos Scientific Laboratory is available from Mound Laboratory, Miamisburg, Ohio 45342. The other producer is Prochem, Post Office Box 555, Lincoln Park, New Jersey 07035. The isotope is available from a number of distributors (for example, see Science - Guide to Scientific Instruments, under Chemicals, Carbon-13 Labeled).

Copyright © 1973 by Academic Press, Inc.
All rights of reproduction in any form reserved.

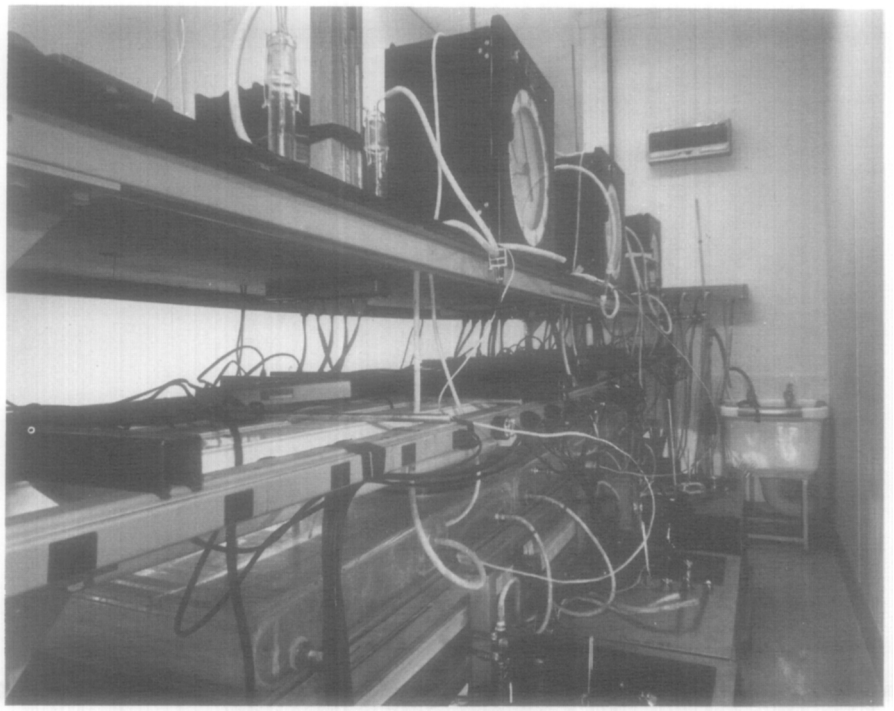

Fig. 1. Incubation chamber. Overall dimensions of the Lucite box are 63 x 290 x 12 cm. Incandescent and fluorescent lights are above and below the chamber which oscillates in the horizontal plane. The cooling baths and some of the fans are visible.

Professor C. D. Raper, North Carolina State University. Published procedures were followed (2,3). Time from planting to maturity is about 75 days. Over the course of these experiments about 8 kg of tobacco leaves were used; these were readily supplied from plants grown in a laboratory bench space of 1 x 3 meters. Leaves cut from the stalks were preincubated 24 hr in the dark at 25° (4) with the stems immersed in half-strength tobacco medium (3). Between 1 and 2 kg of material were used per experiment (10 to 15 leaves).

Incubation:--Incubation with $^{13}CO_2$ was carried out in the apparatus shown in Fig. 1. This consists of three Lucite chambers joined together. An experiment was begun by placing preincubated leaves in the chambers with their stems immersed in medium kept at 20°. The medium temperature in each chamber was controlled automatically and individually through a temperature recorder and controller which operated solenoids regulating the flow of coolant (at -10°)

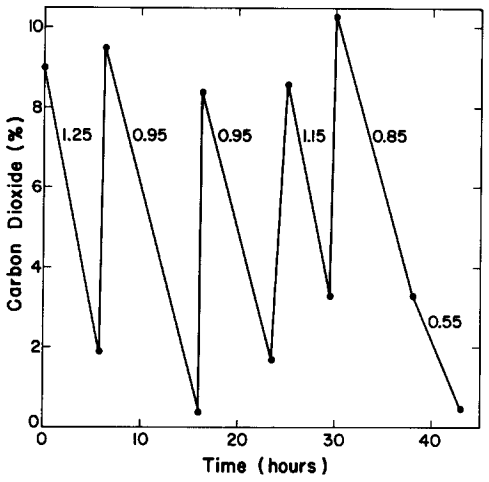

Fig. 2. Rate of consumption of CO_2 by tobacco leaves.

---

from external baths; additional cooling was provided by fans (Fig. 1). When the chambers were loaded and sealed, a slight vacuum was drawn (10 cm of water); then $^{13}CO_2$ was added to a positive pressure of 10 cm of water. Evacuation and loading were repeated until the desired $^{13}CO_2$ concentration was reached (Fig. 2). This procedure caused some 1.5% of the total $^{13}CO_2$ used in the experiment to be removed from the chamber before photosynthesis began. The CO_2 concentration in each chamber was measured with a Harvard Instruments CO_2 conductimetric analyzer, Model 2050. The amount of $^{13}CO_2$ used to fill each chamber was determined by weighing the $^{13}CO_2$ cylinder before and after each chamber was filled. When all chambers contained the desired amount of $^{13}CO_2$, the banks of natural outdoor fluorescent and incandescent lights were turned on, giving 38 and 11 lux at the top and bottom of the chambers, respectively. The chambers were oscillated horizontally to keep the nutrient solution well mixed and to promote efficient cooling. Temperatures of the liquid and gas phases were recorded periodically, along with chamber pressures and CO_2 concentrations. As needed, $^{13}CO_2$ was supplied as described above. Incubation was continued for 40 hr or more to ensure uniform labeling cf the sugars.

This method is adaptable to various scales of operation. By altering

the size of the incubation chamber, the production rate could be varied from a few g to several hundred g of glucose per incubation. We have designed a chamber specifically for leaf incubation with much more efficient cooling and a capacity for two large tobacco leaves and also a 5-liter version of the larger incubation chamber described above. Details of these chambers are available on request.

Isolation of Sugars:--Leaves were removed from the chamber, weighed, frozen in liquid nitrogen, and stored at -20°. The free sugars and starch were extracted essentially as described by Putnam *et al.* (4). Starch was hydrolyzed with trifluoroacetic acid (0.25 N, 8 hr under reflux); glucose, fructose, and sucrose were separated by column chromatography (5,6) and crystallized.

The sugars were identified by enzymatic assay, by gas chromatography of the trimethylsilyl derivatives, and by their nmr spectra. Label uniformity was established by Fast Fourier Transform ^{13}C-nmr spectroscopy (7). The degree of enrichment of carbon-13 in the sugars was determined from the $^{13}C/^{12}C$ ratio in the CO_2 following combustion of the compounds and mass spectrometric measurement of the isotope ratio.

RESULTS AND DISCUSSION

The consumption of $^{13}CO_2$ by tobacco leaves in a typical experiment is shown in Fig. 2. The chamber was refilled with $^{13}CO_2$ four times during the 43-hr incubation. In this experiment, about 0.1 g $^{13}CO_2$ was used per g of fresh leaf. In other experiments, the range was 0.06 to 0.12 g $^{13}CO_2$ per g of leaf. At the end of the experiment, 1.3% of total CO_2 supplied remained in the chamber. The utilization rate of CO_2 changed little until the last few hours of incubation. Changes in carbohydrate yield might have been obtained with longer incubation times or higher temperatures; neither variable was explored. Gas phase temperatures in these experiments reached 35° to 36° at 100% relative humidity, approximating a North Carolina summer day.

Carbohydrate products obtained from eight incubations of tobacco leaves

Table I. Carbon-13 Labeled Carbohydrates Produced by Tobacco Leaf Photosynthesis[a]

Product	Yield[b] g	Yield[b] %
Starch	155[c]	33
Glucose	44	10
Fructose	54	12
Sucrose	21	5

[a]From eight experiments which utilized 8.2 kg of excised leaves and 660 g of $^{13}CO_2$ at 83 atom % carbon-13 (average).

[b]Based on $^{13}CO_2$; corrected for ca. 2% endogenous materials.

[c]Moisture content 14%; hydrolysis and crystallization gave 130 g D-glucose.

are shown in Table I. In a typical experiment, the $^{13}CO_2$ supplied was enriched to 83 atom % carbon-13, and the products contained 81 atom % of the isotope. Nearly 60% of the CO_2 taken up by the leaves was isolated as the four carbohydrates listed in Table I. If all sucrose were hydrolyzed, the total conversion of $^{13}CO_2$ to glucose would be 47%. The nature of the other ^{13}C-containing products in the leaves is unknown.

This investigation of plant leaf systems was begun in order to find ways of producing large quantities of carbon-13 labeled sugars needed for clinical studies in man and for a variety of biochemical experiments. Emphasis was on a high conversion of $^{13}CO_2$ into desired products and on ease and rapidity with which the desired compounds could be obtained when required. The tobacco leaf system, with a 47% incorporation efficiency into glucose in about 40 hr, does well on both counts. Approximately 50 g of crystalline glucose can be produced per week with existing equipment.

A preliminary experiment has been performed on the feasibility of growing potatoes in a carbon-13 atmosphere as a source of labeled glucose (8). The results are difficult to evaluate, since data are not available on the amount

of $^{13}CO_2$ supplied to the plant or the amount of crystalline glucose produced. This method requires continuous exposure of the plant to $^{13}CO_2$ for about 80 days. Equilibrium between the isotopic composition of CO_2 supplied and the gas phase in the growth chamber is achieved after 45 days of incubation (8).

The system described here has proved extremely useful for producing highly carbon-13 labeled sugars in large amounts. The method is applicable to a wide range of glucose requirements and makes this useful substrate available to research organizations at relatively modest cost. In this Laboratory, glucose is produced for about 2 man-hours of labor per g of sugar.

ACKNOWLEDGMENTS

We thank Mr. Victor Chavez, Ms. Darya Turkevitch, and Mr. D. J. Martinez for technical assistance, Dr. T. R. Mills for mass spectrometry, Dr. N. A. Matwiyoff for ^{13}C-nmr spectroscopy, and Professor C. D. Raper, Jr., North Carolina State University, for the tobacco seed and detailed advice on growing the plants. This work was performed under the auspices of the U. S. Atomic Energy Commission.

REFERENCES

(1) Armstrong, D. E., Briesmeister, A. C., McInteer, B. B., and Potter, R. M., Los Alamos Scientific Laboratory Report LA-4391 (April 1970).

(2) Raper, C. D., Jr., Johnson, W. H., and Downs, R. J., Agronomy J. 63:283 (1971).

(3) Raper, C. D., Jr., and Downs, R. T., Agronomy J. (1972), in press.

(4) Putnam, E. W., Hassid, W. Z., Krotkov, G., and Barker, H. A., J. Biol. Chem. 173:785 (1948).

(5) Walborg, E. F., Jr., Ray, D. B., and Ohrberg, L. E., Anal. Biochem. 29:433 (1969).

(6) Jones, J. K. H., and Wall, R. A., Canad. J. Chem. 38:2290 (1960).

(7) Eakin, R. T., Morgan, L. O., Gregg, C. T., and Matwiyoff, N. A., FEBS Letters (1972), in press.

(8) Buchholz, J. R., Christenson, C. W., Eakin, R. T., and Fowler, E. B., unpublished results.