[CONTRIBUTION FROM THE HARRIS RESEARCH LABORATORY, INC., AND NATIONAL RESEARCH COUNCIL]

Biosynthesis of C^{14} Specifically Labeled Cellulose by *Acetobacter xylinum*. III. From D-Glucose-2-C^{14} [1]

BY FRANCIS W. MINOR, GLENN A. GREATHOUSE[2] AND HAROLD G. SHIRK[2]

RECEIVED SEPTEMBER 15, 1954

Cellulose-C^{14} was synthesized by *Acetobacter xylinum* from D-glucose-2-C^{14}. The cellulose had a lower specific radioactivity than the D-glucose-2-C^{14} supplied. The D-glucose obtained by hydrolysis of the cellulose-C^{14} had approximately 60% of the label at position 2 and had significant quantities located at positions 1, 3, 4 and 5, with possibly a trace at position 6.

Introduction

This paper is the third in a series concerned with the ability of *Acetobacter xylinum* to biosynthesize cellulose-C^{14} from variously labeled substrates. A preceding report in this series[3] included results of experiments with D-glucose-1-C^{14} and showed that the cellulose synthesized is primarily labeled in the 1-position (1-C of the glucose from hydrolyzed cellulose) with some randomization of the label among the other carbon positions occurring in some cases. Another report[4] in this series concerned with the cellulose biosynthesized from D-mannitol-1- and/or -6-C^{14} indicated the specific radioactivities of the celluloses formed were lower than those of the substrates furnished the organism. This result may have been caused by a preferential utilization of carbon from unlabeled portions of the substrates. It has also been shown[5,6] that ethanol, as well as sodium acetate, although stimulating the organism to produce greater quantities of cellulose, do not of themselves furnish any carbon for the cellulose. Experiments reported here with glucose-2-C^{14} provide information pertaining to the utilization of still another radioactive substrate and include the label distribution in the resultant cellulose.

Procedures

Culture Conditions.—In these present experiments, the nutrient medium employed for culturing the organism was one found by Shirk[7] to produce relatively high yields of cellulose, compared to previous compositions. It consisted of 1% glucose, 2.25% yeast extract and 1% by volume of 95% ethanol. Duplicate cultures were incubated at 30 ± 0.1°. In each case 20 ml. of the medium was used and 21 microcuries of C^{14} was furnished in the form of D-glucose-2-C^{14}. Inoculation was accomplished with a suspension of actively growing cells prepared from a glucose–agar slant. The crude cellulosic pellicles were harvested at the end of a 7-day incubation period at 30 ± 0.1°. The culture vessel, including accessory apparatus for collecting CO_2 was identical with that described previously.[3]

Analyses.—The procedure for purifying the crude cellulosic pellicles, the determination of C^{14} content in the CO_2 and the cellulose, and the means for locating the label in the glucose from the cellulose were all the same as those previously described.[3]

Results and Discussion

The weight yields, as carbon content and the percentage of C^{14} label in each, of purified cellulose and carbon dioxide, are indicated in Table I. The table shows also, for comparison, the carbon and percentage of label in the D-glucose-2-C^{14} and ethanol that were supplied.

TABLE I

SUBSTRATES, YIELD OF PRODUCTS AND C^{14} LABEL DISTRIBUTION

	Wt., mg. C	Label[a] C^{14}, %	Wt., mg. C	Label[a] C^{14}, %
Substrates				
D-Glucose-2-C^{14}	80.5	100	80.5	100
Ethanol	79.3	None	79.3	None
Products				
Carbon dioxide	104.0	42.2	106.0	39.2
Cellulose	14.4	10.0	14.6	10.2

[a] These columns show percentages of total label in the culture.

The 210 mg. of carbon in the CO_2 from the two cultures exceeds the amount in either the D-glucose-2-C^{14} (161 mg.) or the ethanol (159 mg.). Neither substrate could have formed the total yield of CO_2. Judging from the result of an experiment[3] with D-glucose-1-C^{14}, the percentage of D-glucose carbon oxidized to CO_2 is approximately the same as the percentage of label accounted for in the CO_2. The carbon in this percentage of D-glucose (40%) could account for only a minor portion of the total CO_2. The remainder of the CO_2 came from the ethanol, as this substance is extensively oxidized to CO_2 by *Acetobacter xylinum* even in the presence of D-glucose.[6]

The weight of cellulose carbon (29 mg. total from both cultures) was 18% of the weight of carbon in the D-glucose-2-C^{14}. The percentage of label accounted for in the cellulose, 10.1%, was appreciably smaller, so that its specific radioactivity was low compared to the D-glucose-2-C^{14}. In this respect it was similar to cellulose[3,4] biosynthesized from D-glucose-1-C^{14} and D-mannitol-1-C^{14}.

The label in the cellulose was distributed over the six positions in the glucose units as shown in Table II. The major portion of the label was located at position 2, corresponding to its position in the labeled substrate. Except for position 6, sig-

TABLE II

LOCATION OF LABEL IN GLUCOSE FROM BACTERIAL CELLULOSE

Structure position	Per cent.	Structure position	Per cent.
1	15.6	4	5.0
2	59.8	5	11.5
3	7.1	6	1.1

(1) This series of papers is based on work supported by the Atomic Energy Commission under Contract AT(30-1)-915 with the Harris Research Laboratories.

(2) National Research Council, Washington, D. C.

(3) F. W. Minor, G. A. Greathouse, H. G. Shirk, A. M. Schwartz and M. Harris, THIS JOURNAL, **76**, 1658 (1954).

(4) F. W. Minor, G. A. Greathouse, H. G. Shirk, A. M. Schwartz and M. Harris, *ibid.*, **76**, 5052 (1954).

(5) H. L. A. Tarr and H. Hibbert, *Can. J. Res.*, **4**, 372 (1930).

(6) G. A. Greathouse, H. G. Shirk and F. W. Minor, THIS JOURNAL, **76**, 5157 (1954).

(7) H. G. Shirk, unpublished.

nificant percentages were located at the other positions.

The label at position 2 could result from the direct polymerization of unbroken 6-carbon chains from D-glucose-2-C^{14}. However, the presence of label at other positions shows that much of the labeled cellulose was formed from breakdown products, and, indeed, label in the two position could also have been obtained in this manner.

The mechanism that caused the distribution of label and the comparatively low specific radioactivity in the cellulose are at present unknown. These results, as well as other data reported,[3] suggest that random scissioning of the D-glucose and its degradation products could account for the asymmetrical distribution of label found. Only further investigation, such as a detailed study of the metabolic intermediates, will permit the formation of more definitive conclusions regarding the mechanism of the cellulose biosynthesis.

Acknowledgment.—The authors are grateful to Dr. H. S. Isbell, National Bureau of Standards, Washington, D. C., for the supply of D-glucose-2-C^{14} used in this investigation.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Alkaline Hydrolysis of Scopolamine Methyl Bromide and Other Esters of Quaternary Amino Alcohols[1]

BY ROBERT BRUCE MOFFETT AND EDWARD R. GARRETT

RECEIVED AUGUST 23, 1954

Esters of quaternary amino alcohols have been found to hydrolyze in alkali much more rapidly than the corresponding esters of tertiary-amino alcohols. The rate constants for the hydrolysis of several of these esters were determined. Scopolamine is known to rearrange on hydrolysis giving the basic moiety scopoline. However it was found that scopolamine methyl bromide hydrolyzes without rearrangement to scopine methyl bromide.

A study of the applicability of the acidic dye colorimetric method[2] to the assay of scopolamine methyl bromide[3] (I) indicated that this salt is quite unstable in dilute alkaline solutions.[4] Preliminary titrations showed that this instability was due to extremely rapid hydrolysis of the ester. Only one equivalent of base was consumed and the acid liberated had a pK'_a value which checked with that of tropic acid. There was no evidence of destruction of the quaternary salt. In order to put this study on a more quantitative basis, the rates of hydrolysis were determined for a number of esters of amino alcohols and their corresponding quaternary salts.

Since, even in very dilute alkali, many of these esters hydrolyzed too rapidly for titration of aliquots, an automatic electrometric titrator was used. Samples of the esters in one equivalent of 0.025 N alkali were placed in this instrument which can record pH as a function of time. From such curves values for the reciprocal of the hydroxyl ion concentration were obtained. Plotting these against time gave typical bimolecular rate curves (Fig. 1). The rates for the slower hydrolyzing esters were obtained by mixing the ester with one equivalent of standard alkali and titrating aliquots from time to time. The curves in Fig. 2 and the bottom two in Fig. 1 were obtained by this latter method. It will be noted that the time in Fig. 1 is in minutes while that in Fig. 2 is in hours. Also the two bottom curves of Fig. 1 are repeated as the two top curves of Fig. 2. From these data the bimolecular rate constants (Table I) were calculated.

TABLE I

BIMOLECULAR RATE CONSTANTS[a]

No. (Figs. 1 and 2)	Name of compound	k^a
1	β-Diethylaminoethyl xanthine-9-carboxylate methyl bromide[5]	>100
2	Scopolamine methyl bromide[3]	28
3	Atropine methyl bromide	14
4	2-(2,5-Dimethyl-1-pyrrolidyl)-ethyl phenyl-Δ^2-cyclohexenylacetate methyl bromide[b]	3.1
5	Acetylcholine chloride	2.1
6	Scopolamine hydrobromide	0.27
7	2-(2,2-Dimethyl-1-pyrrolidyl)-ethyl cyclopentyl-n-propylacetate methyl bromide[b]	0.1
8	Atropine	0.08
9	2-(2,5-Dimethyl-1-pyrrolidyl)-ethyl phenyl-Δ^2-cyclohexenylacetate hydrochloride[c]	0.005
10	2-(2,2-Dimethyl-1-pyrrolidyl)-ethyl cyclopentyl-n-propylacetate hydrochloride[c]	<0.001

[a] k (l./mole min.) in 48% ethanol as determined by hydrolysis of 0.025 M solutions of esters in 0.025 N NaOH at 25°. [b] Reported before the Division of Medicinal Chemistry, A.C.S. at Los Angeles, California, March, 1953, Abstracts, p. 8L. [c] R. B. Moffett and J. H. Hunter, THIS JOURNAL, **74**, 1710 (1952).

Although there is a great variation in the rates of hydrolysis of these esters it will be noted that in all cases the methyl bromide quaternary salts hy-

(1) Presented before the Division of Medicinal Chemistry, A.C.S. of Kansas City, Missouri, March, 1954, Abstracts, p. 19M.

(2) F. Durick, J. S. King, P. A. Ware and G. Cronheim, *J. Am. Pharm. Assoc.*, **39**, 680 (1950).

(3) Pamine bromide, The Upjohn Company brand of methscopolamine bromide.

(4) Private communication from Dr. Wm. L. Miller, of our Department of Pharmacology, Mr. Wm. A. Struck and Miss Eleanor J. Scott, of our Analytical Chemistry Laboratory.