METHODS IN CARBOHYDRATE CHEMISTRY

Edited by

ROY L. WHISTLER

III *Cellulose*

METHODS IN
Carbohydrate Chemistry

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Preface

The broad aims of this series have been stated in Volume I. This volume presents many of the specialized methods needed in work with cellulose. The numerous procedures which have gradually evolved over a long period of time are described in a wide literature. Following the central purpose of this series the specific methods assembled here have been prepared by expert investigators. Methods of cellulose chemistry only are presented, and all methods concerned with related plant and microbial polysaccharides are retained for inclusion in Volume V.

Dr. John W. Green alone handled the correspondence with authors and did the initial copy editing. His excellent work and especially his fine cooperation, helpful advice, and wise counsel are greatly appreciated.

ROY LESTER WHISTLER

January 1963

Foreword

In contrast to reactions of the simpler carbohydrates, cellulose reactions are generally heterogeneous and are therefore governed to a great extent by the physical nature of the starting material. The reactions are often incomplete, and the products obtained may have only a portion of their functional groups converted to the desired condition. Therefore quantitative methods are needed to characterize cellulose or its reaction products both chemically and physically, and a great portion of this book is concerned with this aspect.

Only a minimum of background material is given in the various experimental procedures. Therefore the use of a standard text in cellulose chemistry is recommended (*1–4*), or else reference to the more specialized literature cited with each method.

The present volume is restricted closely to the subject of cellulose. Plant and wood chemistry are only briefly considered, as in the isolation of a cellulose. The hemicelluloses are dealt with only as impurities to be removed during the purification of a cellulose. Applications to textile and paper technology have been omitted, and therefore such subjects as mercerization, vulcanization and parchmentization have received scant attention. Again the reader is referred to standard texts for these broader fields.

In recent years the techniques of paper and column chromatography have been developed and several applications of these techniques have been presented.

Various types of cellulose are mentioned as starting materials in the several procedures. These are generally available. The International Commission for Cellulose Analysis has limited quantities of cotton linters and wood pulps (termed "Standard ICCA Samples") for distribution.[1] While the principal use of these materials has been for the development of analytical procedures, they may also be valuable for other aspects of cellulose research.

The writer is indebted to the Administration of the Institute of Paper Chemistry for permission to work on this book as an official project of that organization. He is also grateful to B. L. Browning, E. E. Dickey, H. A. Swenson, K. Ward, Jr., J. Weiner, and L. E. Wise for helpful suggestions. The cooperation of D. G. Sachs in making several of the drawings for the book is appreciated.

[1] Correspondence should be directed to E. E. Hembree, Director, Technical Service, The Buckeye Cellulose Corporation, 2899 Jackson Avenue, Memphis 8, Tenn., for details about the Standard ICCA Samples.

Acknowledgment is made to T. N. Kleinert of the Division of Industrial and Cellulose Chemistry, McGill University, Montreal, Canada, and to F. R. Charles of Industrial Cellulose Research, Limited, Hawkesbury, Canada, for helpful comments on the section concerning xanthation. The help of Derek Horton of Ohio State University concerning certain terminology is appreciated.

The cooperation of M. L. Wolfrom and R. L. Whistler, editors of this series of books, is appreciated, and especially that of J. N. BeMiller, who did the secondary reading of the manuscripts. Finally the writer is very grateful to the authors of the various sections, those whose whole-hearted cooperation has made this book possible.

Appleton, Wisconsin JOHN W. GREEN

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Section I. Preparation of Cellulose

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[1] Purification of Cotton Cellulose

By W. M. Corbett

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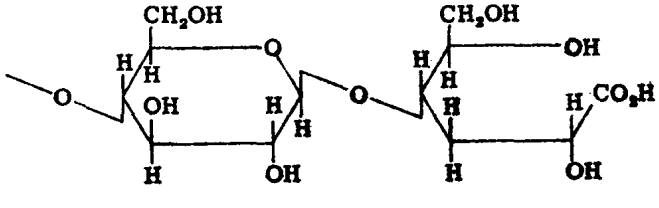
Introduction

Although cotton is the purest form of naturally occurring cellulose, it contains several impurities, such as wax, pectins, and coloring matter. Any method of purification must aim at removing these impurities under conditions which are not sufficient to bring about any fundamental change in the cellulose structure, for example, introduction of groups such as carbonyl and carboxyl. It is essential that the initial cotton is free from extraneous impurities, such as boll particles, stalk, leaf, and so on, since these are resistant to subsequent treatment. Cotton in the form of card sliver is an especially good starting material. Normally, the waxes are removed by extraction with organic solvents, pectins by hot alkali, and coloring matter by bleaching, but, if the conditions of purification are not rigorously controlled, considerable degradation of the cellulose may occur as shown by low viscosity and α-cellulose content. In order to standardize the procedure so that, as far as possible, different workers may use the same cellulose, the Cellulose Chemistry Division of the American Chemical Society (*1*) introduced a method for the preparation of "Standard Cellulose." This method describes the precise manner in which cotton linters were to be extracted with alkali followed by bleaching with hypochlorite. A study of this method by Corey and Gray (*2*) revealed that the bleaching caused considerable degradation and that cellulose of exceedingly high purity can be obtained without bleaching. Present day practice is not to use bleaching agents. The alkaline treatment causes degradation of about 2%, but, in the absence of oxygen, this is of a stepwise nature and causes no detectable decrease in D.P. However, it does modify a few reducing terminal groups (*3*), converting them into D-glucometasaccharinic acid without cleavage from the cellulose molecule. This type of molecule (I) is stable towards further attack by alkali.



(I)

Procedure

Carded cotton sliver, cut into short lengths, is extracted, preferably in a Soxhlet apparatus, for 18 hr. with chloroform, followed by 95% ethanol for a further 18 hr. It is vigorously boiled for 8 hr. in an atmosphere of nitrogen with 1% sodium hydroxide with a liquor ratio of 50 ml. per g., washed with water until free from alkali, and then washed sequentially with acetone, ethanol, and ether. The cellulose is then dried under reduced pressure at 20°.

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[2] Bacterial Cellulose

By Shlomo Hestrin[1]

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Introduction

Processes involving synthesis effected by a growing culture have been used most widely as the means of preparing bacterial cellulose. A typical procedure of this type is detailed. A process involving the use of suspensions of stable washed cells (*1*, *2*) is particularly suitable for cases in which a labeled compound in limited supply has to serve as the substrate of the synthesis.

Procedure

Maintenance of Bacteria

Cellulose-forming *Acetobacter* strains are supplied by Type Culture Collections generally under the name "*Acetobacter xylinum*." Such stock can have a heterogeneous composition and comprise numerous individuals which have lost heritable capacity for cellulose synthesis (*3*, *4*). It has been a frequent experience that repeated passage of such a stock through a quiescent liquid D-glucose medium as described below improves the cellulose-forming capacity significantly. Culture vessels should have a geom-

[1] Deceased, 1962.

etry which permits a membrane formed at the surface of the medium to adhere to the walls of the vessel. Test tubes are entirely suitable from this point of view.

A satisfactory culture medium has the following composition: bactopeptone (Difco) 0.5%, yeast extract (Difco) 0.5%, D-glucose 2.0%, potassium monohydrogen phosphate (anhydrous) 0.1%; pH 7.0. The solutes are stirred in water with heating and the insolubles are removed by passage through an asbestos pad (Seitz). The solution is sterilized by autoclaving it 20 min. at 1.0 atmosphere. Cultures are incubated at 30°. Within a day, a pellicle appears at the surface of the liquid. It contains almost all the cells and formed cellulose. Since cells near the surface are ensured of a good oxygen supply and can grow readily, any shaking which might dislodge the membrane from the surface should be avoided. Oxygen has, however, the undesirable effect of promoting a proliferation of "cellulose-less" mutants (*4*), and these can be expected to be relatively numerous in the surface pellicle. Inoculum for subtransfer is therefore taken from the liquid phase of the medium. Intervals between subtransfers may range, as convenient, from 1 to 14 days. About 1 ml. of inoculum is added to 10 ml. of fresh medium. A culture has been restored to full vigor when it is able to form a readily discernible but transparent surface membrane within 15 hr. following the inoculation.

Conversion of D-Glucose Into Cellulose

Medium of the composition described in the preceding section is distributed into Roux flasks or other suitable covered, flat containers in a liquid layer about 1-cm. thick. The system is sterilized by autoclaving it 20 min. at 1.0 atm. Heat-sterilized aluminum trays are a convenient culture vessel for large-scale work. Inoculum (liquid phase of a 24–48-hr. culture) is added in a proportion of 1:10. During undisturbed 15-hr. incubation at 30°, a transparent membrane appears on the surface of the liquid. With further incubation, the membrane increases in mass and tensile strength and assumes a turbid appearance. The pellicles are ready for harvest after about 5 days. Cellulose yields at this time are generally in the range of 0.5 to 1.0 g. per liter of medium. A second crop of pellicles can be taken several days after the removal of the first crop. Yields as high as 40% on total D-glucose supplied have been accomplished in systems in which the initial concentration of D-glucose was lowered (*5*).

If it is desired to obtain the final product in the form of an unbroken membrane, a process for purification which does not destroy the existing organization is advantageous. A membrane which is colorless and transparent is afforded by prolonged rinsing in flowing water followed by soaking in 4% sodium hydroxide for 7 days at room temperature. To free the

membrane from alkali, it is rinsed successively in water, 0.5% acetic acid, and again in water until the washings are neutral. Membranes prepared in this manner contain 0.2–0.4% of nitrogen by dry weight. After drying in air, the material can be converted into a powder by milling.

If the product can be in the form of dispersed fibers, the procedure described below is employed. The membranes are first allowed to drain and are then disintegrated under water in a Waring blendor. This process yields a cellulose-rich foam over a cell-rich liquid phase. The latter is discarded. The foam is diluted in water and subjected to further blending. The suspension is then passed through several layers of cheesecloth. The fraction on the cloth is scraped off, resuspended in water, and collected by centrifugation (5000 × g for 10 min.). This process is repeated several times until centrifugates are solute-free. Relatively few cells and some insoluble protein contaminating the product are removed by soaking the fiber 3 days in 4% sodium hydroxide at room temperature. Alkali is removed in a stream of water over a suction funnel. The fiber is then rinsed in 0.5% acetic acid and finally in a stream of water until a neutral effluent is obtained. The product tends to settle in water but can be kept in relatively stable suspension in this medium after treatment at a concentration of 20 mg. per ml., first for 15 min. at high speed in a Waring blendor and then for 30 min. in a sonic oscillator (Raytheon, 10 kilocycle).

Characterization of Cellulosic Product

The x-ray diffraction pattern and infrared absorption spectrum of purified bacterial cellulose indicate it to be native crystalline (type I) cellulose, like the α-cellulose of cotton hair (*3*). Analytical recoveries of D-glucose from hydrolyzates of bacterial cellulose have been nearly 90% of theoretical value (*6*). On a basis of yield of trinitrate ester afforded by a bacterial cellulose, this fiber preparation is estimated to be at least 95% cellulosic (*5*). The degree of polymerization of the nitrated derivative, as estimated by viscometry, is about 3000 (*5*, *7*). The electron microscopic image of bacterial cellulose is that of a system of threads sometimes surrounded by an apparently amorphous phase (*8*). It has been found that the threads are composite in structure and can be seen to be built up out of a still thinner unit (*9*) (Fig. 1).

Analysis of Bacterial Cellulose for D-Glucose (*6*)

This method consists of three stages: (1) partial acetolysis of the polysaccharide, (2) acid hydrolysis to D-glucose, and (3) colorimetric determination of the glucose. The combination of acetolysis and hydrolysis is used instead of a single hydrolysis step (see also Vol. III [12]).