[CONTRIBUTION FROM THE FIRST CHEMICAL INSTITUTE, UNIVERSITY OF VIENNA]

# An x-Ray Examination of the Acetates of Glucose, Cellobiose and Cellotriose[1]

BY GERALD J. LEUCK[2] AND H. MARK

Both the theoretical and practical importance of cellulose have caused its structure to be of considerable interest. This interest leads to a desire for as complete knowledge as possible regarding the lattices of the basic molecular fragments and intermediate compounds, the so-called cellodextrins.

The lattices of glucose and cellobiose were not cleared up until a few years ago. This was made possible through success in getting crystals large enough and perfect enough for complete analyses[3] of the elementary cells of the two substances. The purpose of the following investigation was to improve our knowledge in this direction.

In recent years Zechmeister and Tóth,[4] by means of hydrolysis of cellulose, have succeeded in preparing some of the higher cellodextrins, namely, cellohexaose, cellotetraose and cellotriose in addition to the familiar cellobiose and glucose. They have also, by the use of orthodox chemical methods, prepared and studied many derivatives of these presumed fragments of the cellulose molecule. The samples studied in our investigation were some of the materials thus prepared by them.

Preliminary experiments on crystallization of these higher sugars showed that it would be difficult to obtain crystals big enough for investigation even by means of the new microscopic x-ray method of Kratky.[5] The most hopeful products obtained were merely birefringent powders. The acetates of these sugars seemed more promising for x-ray investigation as they showed a tendency to crystallize better. However, of the higher members, we have obtained to date only the acetate of cellotriose in sufficiently suitable crystals to make any kind of x-ray investigation feasible. At the same time the acetates of glucose and cellobiose were crystallized as well as we were able and the cellotriose acetate crystals were studied in comparison with these products.

(1) This work was presented at the Chicago, Ill., meeting of the American Chemical Society, September, 1933.

(2) Present address: The Miner Laboratories, 9 S. Clinton St., Chicago, Ill.

(3) Hengstenberg and Mark, *Z. Krist.*, **72**, 301 (1929).

(4) Zechmeister and Tóth, *Ber.*, **64**, 854 (1931).

(5) Kratky, *Z. Krist.*, **76**, 261 (1930).

### Crystal Preparation and x-Ray Method

In the case of all three substances 96% alcohol was the solvent found most suitable for the purpose of getting individual crystals of the largest size. With the acetates of glucose and cellobiose our best crystals were obtained by making a very dilute solution in hot alcohol. The tube of solution was placed in hot water in a Dewar flask which was in turn enclosed in another larger Dewar flask. With this insulation the solution required several days to cool to room temperature, a condition favorable to good crystal growth.

This method was not advantageous with the acetate of cellotriose because under these conditions the crystals tended always to be fastened together in unoriented clusters. The best method used for this substance was to make a concentrated alcohol solution, practically saturated at boiling temperature, and allow to cool down to room temperature without insulation. When, after a few minutes, the product had become a stiff mass, it was diluted with several times the volume of cold alcohol and shaken gently to disperse the mass. Thus a comparatively large proportion of single crystals resulted.

The crystals of all three substances were isolated by washing with small amounts of very cold alcohol, either as they adhered to the walls of the crystallization tube, or on a microfilter and then dried in a vacuum desiccator.

The best glucose acetate crystals obtained were several mm. long, 0.2 mm. wide and 0.02 mm. thick. The dimensions of the cellobiose acetate crystals were less than one-tenth as large, and those of the cellotriose acetate were still smaller. The x-ray micro camera could no doubt have been used to advantage with single crystals of the glucose acetate, but since the other crystals, particularly those of the cellotriose acetate, gave evidence of being too small for single crystal work with even this special apparatus, work along this line was discontinued and was substituted by special technique for orienting the directions of groups of crystals. This gave some hopes of success with all three substances and had the advantage that an ordinary x-ray camera with its less difficult possibilities could be used. Bundles of glucose acetate crystals were built up with orientation so exact in all three directions that the x-ray photographs obtained from them approached in accuracy those which would be obtained from single crystals of the size of the bundles. This was accomplished by means of painstaking manipulation under a microscope. Enough of the board-shaped crystals were pasted together (saliva as the adhesive) so that the thickness of the bundle (0.1 to 0.2 mm.) approached the width of a crystal. Particular care was necessary to keep the orientation accurate in all three directions.

The same method of orienting bundles was used with cellobiose acetate, but because of the much smaller size of the crystal it was less successful here. In only one direction, that of the needle axis, did the accuracy of the orientation approach that with glucose acetate, and

in this case it gave a photograph similar to those obtained from bundles of fibers.

The orientation of cellotriose crystals by this method was even less successful because of the still smaller crystals. The use of a trace of mineral oil instead of saliva as an adhesive was more successful but the photograph was still largely Debye–Scherrer rings such as are obtained from a haphazardly arranged birefringent crystalline powder. Nevertheless there were a few interference spots unobscured by the rings which could be interpreted and calculated as layer lines.

As an additional method of orienting the cellotriose acetate crystals, a quantity of them was subjected to a pressure of about 5000 atmospheres, a method which is known to give considerable orientation in many cases.[6] The pressure prepared sample gave largely a ring photograph like the other product from cellotriose acetate, but it likewise gave some unobscured interference spots, and the spots varied in arrangement depending upon whether the direction of rotation in taking the photograph was perpendicular or parallel to the direction of pressure used in preparing the sample. These spots were likewise capable of interpretation and calculation as layer lines, and of correlation with those obtained from the other cellotriose acetate sample.

Monochromatic x-ray from copper, $\lambda = 1.54$ Å., was used in this work.

With the exception of some Debye–Scherrer ring photographs made with cellotriose acetate for comparison purposes, layer line rotation photographs, made on a cylindrical film in a cylindrical camera of 57.2 mm. working diameter, were used exclusively in this work.

The densities of the three substances investigated were estimated by the method of floating the air freed crystals in salt solution, determining the densities of these solutions, and considering these densities to be those of the crystals.

## Discussion of Results

| | Identity periods calculated from Polanyi formula, $n\lambda/\sin \mu_n$ | | | Molecules per unit cell |
|---|---|---|---|---|
| | a, Å. | b, Å. | c, Å. (needle axis) | |
| Glucose pentaacetate | 24.3 | 14.9 | 5.65 | 4 |
| Cellobiose octaacetate | *21* | *15* | 5.7 | *2* |
| Cellotriose undecaacetate | *29–30* | *15* | *5.7* | *2* |

As explained below, the values italicized in the table were obtained either by indirect calculation or from questionable experimental data, or from a combination of the two. These suggested values are accordingly tentative speculations rather than established facts, and both they and deductions based upon them are proposed in that sense only.

Of the factual nature of the other values in the table there cannot be much doubt. The three directions of rotation by means of which the three identity periods of glucose acetate were determined were perpendicular to one another, which indicates an orthorhombic crystal structure. That the identity periods cannot incline far from perpendicular to form a structure anything more than very slightly monoclinic or triclinic is demonstrated further by the fact that all three directions formed normal layer line photographs. Because the samples used were composite crystals built up from a number of crystals to take the place of a single crystal, it is highly improbable that they could have given normal single crystal layer lines if the identity periods had not been substantially perpendicular to each other. Furthermore, these layer-line photographs of built-up crystals were highly rational: the plane spacings, calculated according to the Miller indices formula of the orthorhombic crystal system, checked so closely and completely with the experimentally found plane spacings (not given here), that the space groups to which the orthorhombic crystal could be assigned were reduced down to three low symmetry possibilities.

Since the volume of the elementary cell in the orthorhombic system is the product of the three dimensions, that of glucose acetate is 24.3 Å. $\times$ 14.9 Å. $\times$ 5.65 Å. = 2046 Å.[3] The density is 1.274 so that the weight of the elementary cell is $2046 \times 10^{-24} \times 1.274$ or $2608 \times 10^{-24}$ g. The molecular weight of glucose pentaacetate times the weight of one hydrogen atom is $390.3 \times 1.65 \times 10^{-24}$ or $643 \times 10^{-24}$ g. The division of $2608 \times 10^{-24}$ by $643 \times 10^{-24}$ gives 4.06, which is within the limit of error, so that it shows the number of molecules in the elementary cell to be four.

As given in the table, the remaining definitely established identity period, which is 5.7 Å. along the needle axis, belongs to cellobiose acetate. This value checks within the limit of error with the corresponding value of the identity period along the needle axis of glucose pentaacetate and the two photographs show a strikingly characteristic similarity also in such respects as corresponding regions of maximum and minimum intensities. The only definitely recognizable difference is a slight variation in the positions of the plane spacing flecks. Because of the inexactly oriented bundle nature of the cellobiose acetate sample, only the flecks on the equator near the undeflected ray center are capable of definite calculation. If one assumes that the structure of the cellobiose acetate is practically identical with that of glucose acetate except that the $\alpha$-axis spacing is about 21 Å. instead of 24.3 Å., the spacing flecks which are sharp enough and significant enough to be

(6) Herzog and Jancke, *Z. angew. Chem.*, **59**, 385 (1921).

evaluated (data and calculations not given here), agree reasonably well with orthorhombic crystal structure theory, and the assumption does not interfere with the remaining general similarity of the two photographs. Moreover, if we assume that there are 2 molecules in the unit cell and calculate from the molecular weight 686, the known identity period 5.7 Å. and the determined density of 1.275 in a reverse order from that above with glucose acetate, the product of the other two dimensions must be about 310 Å.[2] The product of 15 Å. and 21 Å. is about 310 Å.[2] also.

It was attempted to get layer line photographs of identity periods in the other two perpendicular directions, but the orientation could not be carried out well enough to make the photographs reliable in interpretation. The photographs gave no evidence in conflict with the above deductions, and there were a number of interference flecks which appeared to check with various orders of the two suggested identity periods, but these flecks tended to occur singly instead of in pairs as in the case of the cellotriose acetate discussed below, and so no reliance as evidence is given them.

In the case of cellotriose acetate the assumption of two molecules in the elementary cell leads to results in agreement with those suggested for the other two substances. The data available from attempted layer line photography indicate a short identity period of about 5.7 Å. as with the other two substances, and this is further substantiated by the two regions of the beginning of heavy rings on Debye–Scherrer photographs of cellotriose acetate to correspond to the first and second orders of this identity period.

The data from the questionable layer line photographs of cellotriose acetate also indicate the possible presence of two other identity periods of about 15 Å. and 30 Å. These three spacings are compatible with an assumed two molecules to the cell and a determined density of 1.278 and the molecular weight of 966 when considering the molecular volume. In fact, if one assumes that the two shorter spacings of cellotriose acetate are identical with those found for glucose acetate, 5.65 Å. and 14.9 Å., this molecular volume calculation gives the third identity period the value of 29.5 Å.

The shortest identity period of glucose acetate was found to be along the needle axis and the data on cellobiose acetate indicate that in all probability it has a similar structure in this respect. The attempted layer line photographs of cellotriose acetate in which the identity period along the needle axis should tend to predominate favor the shortest identity period of about 5.7 Å. more than those photographs in which identity periods perpendicular to the needle axis should tend to predominate. This suggests that the molecular chains in all three substances lie perpendicular to the needle axis, an interpretation which is in reasonable agreement with the general knowledge of chains and long spacings in ordinary crystals.[7]

If one wishes to speculate further upon the directions of the chain spacings of the acetates of cellobiose and cellotriose, the addition of atomic radii and usual intermolecular space requirements indicates that, with both compounds, the longest identity period is preferable to the 15 Å. identity period as the chain spacing. Inasmuch as there are four molecules in the unit cell of glucose acetate and the molecular chains are shorter than those of the other two compounds, there would be no justification for assuming on the basis of space requirements that its longest identity period represents the direction of the chain spacing. Yet to make this assumption gives a satisfactory picture of these compounds as derivatives of fragments of a chain cellulose molecule. In this case the longest identity period of glucose acetate should be approximately halved in order to get all compounds on the same basis of two molecules per unit cell. Then, in addition to two shorter spacings of approximately 5.7 Å. and 15 Å. common to all these compounds, there would be a chain spacing which increases steadily with the number of glucose residues in the molecule: 24.3/2 Å., 21 Å., 29–30 Å.

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## Summary

1. A special technique for building crystals of

(7) Müller and Schearer, *J. Chem. Soc.*, **123**, 3156 (1923); La Tour, Thesis, University of Paris, 1932.

the acetates of glucose, cellobiose and cellotriose has been developed.

2. Identity period measurements and calculations of these built-up crystals have been obtained.

3. Speculations upon the spacings of these compounds in relation to the cellulose molecule have been presented.

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[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

# Semicarbazone Formation in Sixty Per Cent. Methyl Cellosolve[1]

BY FRANK H. WESTHEIMER[2]

In a recent paper Conant and Bartlett[3] determined the kinetics and equilibria of semicarbazone formation in aqueous solution. They found that the bimolecular addition

$R_1R_2CO + NH_2NHCONH_2 \rightleftharpoons R_1R_2COHNHNHCONH_2$

is the rate determining step, while the dehydration

$R_1R_2COHNHNHCONH_2 \rightleftharpoons R_1R_2C{=}NNHCONH_2 + H_2O$

follows instantaneously. The reaction is acid catalyzed and the dependence of the rate of condensation on acidity was, in two cases, worked out. The present study was undertaken to determine the changes caused by operating in a semi-aqueous solvent. The one chosen was a mixture of sixty per cent. by volume of methyl cellosolve and forty per cent. of water.

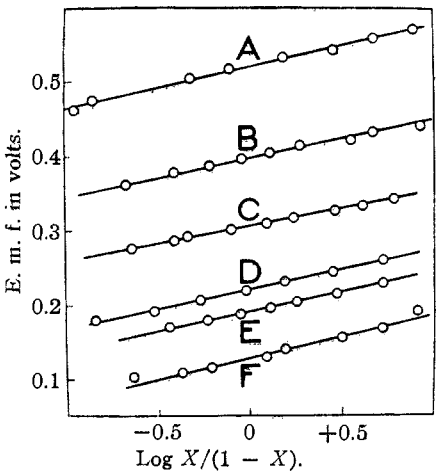


Fig. 1.—A, tributylamine-HCl; B, *p*-nitrophenol-NaOH; C, acetic acid-NaOH; D, dimethylaniline-HCl; E, monochloroacetic acid-NaOH; F, dichloroacetic acid-NaOH.

Since the reaction in water is dependent upon acidity, it was necessary to know the comparative acidities of various buffer solutions in the new solvent. The first part of this paper deals, then, with hydrogen ion determinations, the second with the kinetics of semicarbazone formation, in sixty per cent. methyl cellosolve.

## Acidities

The acidities were measured by electrometric titration. This method, as generally applied, measures the e. m. f. set up between an aqueous calomel cell and the buffer solution in question in the particular non-aqueous or semi-aqueous solvent used.[4] That this method is not subject to error due to the mixed solvent junction has recently been established by Halford.[5]

The antimony electrode so successfully employed by Halford in his butyl carbitol–water mixture was chosen for this work. The results of the titrations gave a series of buffers covering a larger range of acidities than that needed in the kinetic work. Figure 1 is a graph of the titrations, where the e. m. f. is plotted against log $X/(1-X)$ ($X$ is the fraction of the acid neutralized). All the curves have practically the same

TABLE I

STRENGTH OF ACIDS, COMPARED TO ACETIC ACID, IN 60% METHYL CELLOSOLVE

| Acid | $\Delta pK$ Water[6] | 60% Methyl cellosolve |
|---|---|---|
| Chloroacetic | −1.9 | −2.2 |
| Dichloroacetic | −3.5 | −3.5 |
| *p*-Nitrophenol | 2.5 | 1.6 |
| Semicarbazonium ion | −1.1 | −2.4 |
| Tributylammonium ion | 5.2 | 3.9 |
| Dimethylanilinium ion | 0.3 | −1.7 |

(1) Trade name for glycol monomethyl ether.

(2) John Harvard Fellow, 1933–1934.

(3) Conant and Bartlett, THIS JOURNAL, **54**, 2881 (1932).

(4) (a) Hall and Conant, *ibid.*, **49**, 3047 (1927); (b) Conant and Hall, *ibid.*, **49**, 3062 (1927); (c) Hall and Werner, *ibid.*, **50**, 2367 (1928); (d) Conant and Werner, *ibid.*, **52**, 4436 (1930); (e) Halford, *ibid.*, **53**, 2944 (1931); (f) Bright and Briscoe, *J. Phys. Chem.*, **37**, 787 (1933).

(5) Halford, THIS JOURNAL, **55**, 2272 (1933).

(6) The aqueous values for the acids are taken from the "I. C. T." The value for the semicarbazonium ion was determined by Conant and Bartlett. The $pK$ of dimethylanilinium ion and tributylammonium ion were taken from Hall and Sprinkle, THIS JOURNAL, **54**, 3469 (1932).