THE POSITION OF FREE HYDROXYL GROUPS
IN ACETONE-SOLUBLE CELLULOSE ACETATE

Dissertation
Presented in Partial Fulfilment of the Requirements
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Graduate School of The Ohio State University

by
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The Ohio State University
1955

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[Signature]
Advisor
Department of Chemistry
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I am very grateful to the DuPont Corporation for their generous fellowship.

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THE POSITION OF FREE HYDROXYL GROUPS
IN ACETONE-SOLUBLE CELLULOSE ACETATE

I. INTRODUCTION

Prior to this century, cellulose acetate found only limited application in industry. However, during the past forty or fifty years, cellulose acetate has become an increasingly important commercial product. One of the reasons for the increasing interest is the development of a commercial product which is soluble in acetone. Previously, the principal solvent for cellulose acetate was chloroform, which is relatively expensive. The use of inexpensive acetone as a solvent has enabled the industry to grow to large proportions.

Chloroform-soluble cellulose acetate is prepared by acetylation of cellulose to a triacetate. When this triacetate is partially deacetylated, a product is formed which is soluble in acetone. The extent of deacetylation is very important, since acetone solubility appears to be limited to a deacetylated product with a degree of substitution between 2.1 and 2.7 acetate groups per anhydroglucose unit.

Without further evidence, one might be led to believe that the primary cause of acetone solubility is the change in polarity resulting from an increased ratio of free hydroxyls to acetate groups. However, this explanation is insufficient. By partial acetylation of cellulose, a cellulose acetate having a degree of substitution between the limits 2.1 - 2.7 can be prepared which is insoluble in acetone. Thus we see how a product prepared by deacetylation of a cellulose triacetate and
a product prepared by partial acetylation of cellulose can have the same
degree of substitution but have different solubility properties. Sev-
eral explanations have been proposed to explain this.

One theory is that acetone solubility can be attributed to degrada-
tion of the polymer chain into smaller units. This theory does not
appear adequate, since acetone-soluble cellulose acetate can be reacetyl-
ated to an acetone-insoluble triacetate.

Another theory is that the difference in solubility of the two pro-
ducts, prepared by the two different methods but with the same degree
of substitution, can be related to differences in the position of the
free hydroxyl group in the anhydroglucose unit. In cellulose, the an-
hydroglucose unit has three positions available for substitution. Of
these, two are secondary and one is primary. The manner in which these
are substituted could certainly make a difference in properties. It
has been suggested that the position of the free hydroxyl groups in
acetone-soluble cellulose acetate are not the same as those of the cel-
lulose acetate with the same degree of substitution prepared by partial
acetylation of cellulose.

The latter theory appears more likely than the former. It is be-
lieved that cellulose acetate prepared by partial deacetylation of cel-
lulose triacetate would be more homogenous than cellulose acetate pre-
pared by partial acetylation. That is, the free hydroxyl groups of
acetone-soluble cellulose acetate would be distributed in a random
fashion among the three positions in the anhydroglucose unit. Although
the literature evidence supports this random distribution, the work
reported to date is of an inconclusive nature. The conclusions were mostly drawn from indirect measurements.
II. STATEMENT OF THE PROBLEM AND PLAN OF ATTACK

The general purpose of the present investigation was to further the study of the structure of acetone-soluble cellulose acetate. It was proposed to replace the free hydroxyl groups in acetone-soluble cellulose acetate with tertiary amines and then to isolate and identify the products resulting from the hydrolysis of this amino cellulose. The identification of an amino hexose with the amine group in a certain position would be positive evidence that that position existed as a free hydroxyl group in the original cellulose acetate.

The following plan of attack was proposed:

Commercially available acetone-soluble cellulose acetate was to be esterified with p-toluenesulfonyl chloride. Then the tosyl ester groups would be in the position originally occupied by the free hydroxyl groups. This tosylated cellulose acetate was then to be reacted with a secondary amine. The amine would then replace the tosyl ester groups, leaving a tertiary amine in the position originally occupied by the free hydroxyls. The acetate groups would be removed, leaving free hydroxyl groups. The reaction scheme can be written in the manner shown on page 5.

In this reaction sequence it would be necessary that no acetyl migration occur, that a good tosylation of the free hydroxyls be effected, and that complete replacement of tosyl by amine be carried out.
Proposed Reaction Scheme

Cellulose residue of acetone-soluble cellulose acetate

\[ \text{II} + 7\text{H} - \text{N}^\text{R} \rightarrow \]

\[ \text{III} \]

\[ \frac{\text{H}^+}{\text{H}_2\text{O}} \]

*Ts: \( \text{CH}_3\text{SO}_2^- \)
After the tosylation and amine exchange reactions have been completed, it would be necessary to hydrolyze the amino cellulose down to the monosaccharide derivatives. In all probability, the replacement of tosyl groups by amino groups proceeds with inversion of the carbon atom. Therefore, we would expect the formation of one, two, or three of the following compounds, besides D-glucose.

(a)  
\[
\begin{align*}
\text{HO-C} & -
\text{H-C-OH} \\
\text{R-NC-H} & \\
\text{HO-C-H} & \\
\text{H-C-OH} & \\
\text{CH}_2\text{OH} & 
\end{align*}
\]

(b)  
\[
\begin{align*}
\text{HO-C} & -
\text{H-C-OH} \\
\text{H-C-N\text{'-R}} & \\
\text{H-C-OH} & \\
\text{H-C-OH} & \\
\text{CH}_2\text{OH} & 
\end{align*}
\]

(c)  
\[
\begin{align*}
\text{HO-C} & -
\text{H-C-OH} \\
\text{H-C-N\text{'-R}} & \\
\text{H-C-OH} & \\
\text{H-C-N\text{'-R}} & \\
\text{CH}_2\text{N\text{'-R}} & 
\end{align*}
\]
It was assumed with reasonable certainty that very few di- and triamino compounds would be obtained.

After hydrolysis of the polymer, the individual monosaccharides would be separated by chromatography and identified. The presence of compounds (a), (b), and (c) would indicate that the free hydroxyls are distributed in a random manner. The presence of only one or two of the amino monosaccharides would indicate that the free hydroxyls occupy only certain positions in the anhydroglucose unit.

If the conditions mentioned above could be obtained, this method of approach should be very fruitful. It would not depend upon the interpretation of tosylation rate curves (discussed later).
III. HISTORICAL SURVEY

A. Acetylation of Cellulose

The first reported acetylation of cellulose was by Schützenberger (1), who heated cellulose with acetic anhydride in a sealed tube at 180°C. This product was apparently degraded, due to the drastic conditions used. Later investigators found that the use of catalysts such as sulfuric acid would enable the reaction to be carried out under milder conditions (2). Also, salts such as zinc chloride and sodium acetate were found to be good catalysts for acetylation (3), (4).

(1) P. Schützenberger, Compt. rend., 61, 485 (1865)

(2) Franchimont, Compt. rend., 89, 711 (1879)


Later, very strong catalysts such as perchloric acid were used to obtain cellulose triacetate (5).

(5) C. J. Malm, U.S. 1,645,915 (Oct. 18, 1927)
It is believed that the role of sulfuric acid is not entirely catalytic (6), (7).


More recently, trifluoroacetic acid has been reported to be a good acetylation catalyst (8).


B. Acetone-Soluble Cellulose Acetate

In the instances discussed above, the cellulose triacetate (i.e. \(94.8\%\) acetyl) may be formed with varying degrees of degradation, depending upon the conditions of reaction. Because of its insolubility in cheap commercial solvents, only limited use has been found for this triacetate. The best solvents are glacial acetic acid and a 95% mixture of chloroform and methanol. It is not possible to prepare an acetate of cellulose which is soluble in acetone by interruption of the acetylation process (9).

The preparation of an acetone-soluble cellulose acetate was first described by Miles (10). In this process, aqueous acetic acid is added to the acetylation mixture after the triacetate has been formed. This reaction mixture is then heated at 50°C, for several hours. Although Miles did not recognize it, part of the acetyl groups are removed. Today, acetone-soluble cellulose acetate is made in essentially the same manner.

C. Previous Work on the Location of the Free Hydroxyl Group in Acetone-Soluble Cellulose Acetate.

In 1934, Sakurada and Kitabatake (11) found that only one-third


of the free hydroxyl groups in acetone-soluble cellulose acetate would react with triphenylmethyl chloride. If only the primary hydroxyl groups will react with this reagent, then one-third of the free hydroxyls must be primary.

When Cramer and Purves (12) reacted p-toluenesulfonfyl chloride

(12) F. B. Cramer and C. B. Purves, J. Am. Chem. Soc. 61, 3458 (1939)

with acetone-soluble cellulose acetate containing .56 free hydroxyl
groups per anhydroglucose unit, they found that one-third of the hydroxyl groups were rapidly tosylated. When the completely tosylated product was reacted with sodium iodide in acetone, .197 moles of the tosyl groups were replaced by iodine. They believed that only the tosyl groups in the primary position were replaced. This work was later extended by Gardner and Purves (13). These workers removed samples at regular intervals from the tosylolation mixture and reacted them with sodium iodide in acetone. Most of the tosyl groups were replaced by iodine in samples containing up to .197 tosyl groups per anhydroglucose unit.

In samples containing more than .197 tosyl groups per anhydroglucose unit, the iodination reaction is incomplete. This agrees with the previous results. When reaction rates were run on the tosylolation, it was found that one-third of the free hydroxyls were rapidly tosylated, .139 free hydroxyls per anhydroglucose unit were tosylated fairly rapidly and .223 free hydroxyls reacted very slowly. By comparison with tosylolation experiments on ethylcellulose, they assigned the above positions to the 6, 2, and 3 positions respectively (14). The first order rate constants assigned to positions 6, 2, and 3 were 23.4, 2.16 and .106.

In the work of Purves and his co-workers, considerable reliance was placed on the assumption that only the primary tosyl ester would
undergo iodination. Upon repeating this work, Malm, Tanghe, and Laird (15) found that the degree of substitution of iodine increased with increasing degree of substitution of tosyl groups, even after all the primary tosyl groups had been iodinated. Also, tosylated cellulose could be iodinated to a derivative which contained more than one iodine atom per anhydroglucose unit. Apparently, the assumption that only primary tosyl groups can be iodinated is not valid.

Redfarn and Boyle (16) attempted to repeat Purves' work and could not arrive at similar results. They concluded that very little is known about the position of free hydroxyl groups in acetone-soluble cellulose acetate. While this may be an unjustified statement, certainly a good deal more could be learned about this problem.

D. Replacement of Tosyl Groups in Cellulose Derivatives by Amines

The reaction between tosylated cellulose and ammonia was first observed by Karrer and Wehrli (17).

They heated tosyl cellulose containing 1.9% sulfur with aqueous
ammonia at 100°C. for one hour. A product containing 1.3% sulfur and
.7 - .8% nitrogen was obtained.

Sakurada (18) heated monotosyl cellulose with alcoholic ammonia
in a sealed tube for ninety hours. After hydrolysis of the unreacted
tosyl groups, the unsubstituted cellulose was extracted with Schweizer's
reagent. The resulting amino cellulose contained one amino group for
every two anhydroglucose units. Similar results were obtained by re-
acting aniline with monotosyl cellulose in glycerin.

Hess and Ljubitsch (19) heated tosyl cellulose containing 13.97%
sulfur with diethylamine in a sealed tube. The product contained 9.94%
sulfur and 1.41% nitrogen.

In 1937, Ssokolowa (20) heated tosylated cellulose (3.56% sulfur)
with 25% aqueous ammonia and obtained a product which contained 2.64%
sulfur and .8 - 1.0% nitrogen.

Another example of amination of tosyl groups is discussed by
Gardner (21) who also obtained poor results on replacement of tosyl
groups. Some workers were even led to believe that no actual replace-
ment of tosyl groups occurred. The nitrogen content was attributed to adsorbed amine. While this is undoubtedly not true, certainly the reaction has occurred to only a slight extent.

However, a quantitative replacement of tosyl groups by amine groups was almost achieved by Haskins (22) in 1939. By allowing n-amylamine to react with a cellulose derivative containing 1.25 tosyl and .35 methyl groups per anhydroglucose unit, a product was obtained which contained .99 n-amylamino and .23 tosyl groups per anhydroglucose unit.

The amination reaction was applied to tosylated secondary cellulose acetate (degree of substitution of tosyl = .445, and degree of substitution of acetyl = 1.603) by Weinstein (23). The tosylated cellulose acetate was reacted with isobutylamine at room temperature for fifteen days. The reaction formed a gel which was broken up by the addition of .5 N sodium hydroxide. Only 1.66% nitrogen was found in the product which contained no sulfur and no acetyl. The theoretical amount of nitrogen would be 3.39%, if the degree of substitution of isobutylamine were .445. It is believed that the tosyl and acetyl groups were hydrolyzed by the .5 N alkali.

From these considerations, it was apparent that a more suitable replacement of tosyl by amine must be effected if the proposed plan of
study was to be of value.

E. Chromatography of Sugars

Until very recently, the separation of a mixture of sugars into the various constituents was extremely difficult. Often the mixture was simply analyzed for total reducing sugar and very little attempt was made to distinguish one component from another.

Separation of sugars by chromatography was introduced by W. S. Reich (24) in 1939. He found that the p-phenylazobenzate esters of D-glucose and D-fructose could be separated on a column of silica gel developed with a mixture of benzene and petroleum ether.

Following this publication, there was extensive interest shown in finding suitable derivatives and adsorbents which would extend the use of chromatography to more carbohydrates. One leader in this field is M. L. Wolfrom. Wolfrom and co-workers (25) were able to separate many of the sugar acetates on a column of Magnesol (2 MgO·5 SiO₂) and Celite using benzene-ethanol as the developer. Later they were able to separate many of the free sugars on a column of Florex XXX (hydrated sodium aluminum silicate) and Celite by developing with various mix-
tures of alcohol and water (26). Wolfrom and co-workers (27) also


described the use of Silene EF (hydrated calcium acid silicate) to separate free sugars and also sugar acetates.

Recently Whistler and co-workers (28) have used charcoal as a

(28) R. L. Whistler and Chen-Chuan Tu, J. Am. Chem. Soc., 74, 3609 (1952)

chromatography adsorbent to separate the components of the mixture resulting from the hydrolysis of xylan.

Perhaps one of the greatest contributions to the problem of the identification of individual sugars in a mixture came as the result of the work by Partridge (29). He was the first to apply paper chromatography to the field of carbohydrates. Using this method, he was able to separate many sugars and determine their $R_f$ values (30). Paper chromatography has become an increasingly important tool to the carbohydrate chemist.

(29) S. M. Partridge, Nature, 158, 270 (1946)

(30) $R_f$ value = distance moved by the sugar

distance moved by the solvent
It appeared logical that if sugars can be separated on a small scale by paper chromatography, they could be separated on a larger scale using a column packed with cellulose. This was tried and found to be very successful (31). Closely related sugars are now being quantitatively separated by means of cellulose columns. An example is the separation of the alpha and beta anomers of methyl hexosides (32).


(32) I. Augestad, E. Berner, and E. Weigner, Chemistry and Industry, 1952, 376

Another valuable chromatographic method for the separation of sugars is the use of ion exchange resins. Khym and Zill (33) were able to separate many mono- and disaccharides on such strong anion exchanges as Dowex 1 by making the borate complexes of the sugars. Even such closely related sugars as D-glucosamine hydrochloride and D-galactosamine hydrochloride can be separated on a column of Dowex 50 (34).


There are numerous instances in the literature describing the
chromatographic separation of specific sugars that would have been all but impossible thirty years ago. In general, the methods used are essentially those that have been described.
IV. DISCUSSION OF EXPERIMENTAL RESULTS

A. Acetyl Analysis of Cellulose Acetate

In order to determine the extent of tosylation, it was necessary to know the degree of substitution of acetyl in the original cellulose acetate. The material was received with an acetyl analysis of 39.4% (35). The sample was sent to three analytical houses for analysis. The analysis of this compound by these companies is listed below.

<table>
<thead>
<tr>
<th>Company A (36)</th>
<th>Company B (37)</th>
<th>Company C (38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.30</td>
<td>45.29</td>
<td>36.9</td>
</tr>
<tr>
<td>34.04</td>
<td>44.67</td>
<td></td>
</tr>
<tr>
<td>36.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(36) Clark Microanalytical Laboratory, Urbana, Illinois

(37) Geller Microanalytical Laboratories, Hackensack, New Jersey

(38) Huffman Microanalytical Laboratories, Wheatridge, Colorado

Obviously, these results could not be trusted. In order to determine the percent of acetyl accurately, an analytical procedure sim-
ilar to the Ost method was carried out (39).


The results of three acetyl analyses carried out on the cellulose acetate were:

a) 37.89%,

b) 37.90%, and

c) 38.05%.

The average acetyl is 37.95%. The number of acetyl groups per anhydroglucose unit may be calculated by using the formula:

\[
\text{Degree of substitution of acetyl} = \frac{1.621 \times \% \text{ acetyl}}{43.044 - 204 \times \% \text{ acetyl}}
\]

Using 37.95% acetyl, the degree of substitution of the original cellulose acetate is 2.27, leaving .73 free hydroxyl groups.

B. Tosylation of Cellulose Acetate

The cellulose acetate was tosylated in pyridine with p-toluene-sulfonyl chloride at 100°C. for ten days. Cramer and Purves (12) found that the tosylation reaction reached a maximum between four and ten days. Under these conditions, Purves found that there was no acetyl migration even with partially acetylated glucose (13). In the present work, the products were worked up and analyzed for sulfur, nitrogen, chlorine, and acetyl. The nitrogen may be present in the form of adsorbed pyridine. Any chlorine present would result from
chlorination by pyridine hydrochloride. At the low reaction temperature used, the amount of chlorine in the product was negligible. Also, the amount of nitrogen was extremely small. The sulfur content varied from 4.82 to 5.0%, and the acetyl content from 28.04 to 31.85%. The degree of substitution of tosyl and of acetyl can be calculated by solving simultaneous equations.

Let $x$ = number of acetates per anhydroglucose unit

Let $y$ = number of tosylates per anhydroglucose unit

Sulfur = 5.00%,

Acetyl = 28.04%.

1) Molecular weight of one anhydroglucose unit =

$$111.12 + 59.04 x + 171.2 y + (3 - x - y) 17.01$$

2) Molecular weight of one anhydroglucose unit =

$$\frac{32.05 y}{.0500} = \frac{43.04 x}{.2804} = 641.0 y = 153.5 x$$

From equation (2) we obtain $y = .2395 x$.

Now substitute this value for $y$ in equation (1), and substitute $153.5x$ for the left side of the equation.

This gives:

$$153.5 x = 111.12 + 59.04 x + 141.00 x + 51.03 - 17.01 x - 4.07 x$$

By collecting terms we obtain:

$$74.5 x = 162.15$$

and $x = 2.175$ = degree of substitution of acetyl

$y = .521$ = degree of substitution of tosyl.
From these calculations it is apparent that not all of the free hydroxyl groups have been tosylated. The value of 71.4% tosylation agrees well with the value of 73.0% obtained by Purves (12). If the reaction is prolonged, or if the reaction temperature is increased, the replacement of tosyl by chlorine becomes appreciable.

For the present work, it would be much more desirable if complete tosylation could be effected, but the advantages would not justify the undesirable side reactions which would occur. The results given below are from typical products.

### TABLE I

**SUMMARY OF TOSYLATION REACTIONS**

<table>
<thead>
<tr>
<th>Reaction No.</th>
<th>% Sulfur</th>
<th>% Acetyl</th>
<th>D.S.* Tosyl</th>
<th>D.S.* Acetyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.82</td>
<td>31.85</td>
<td>0.535</td>
<td>2.636</td>
</tr>
<tr>
<td>11</td>
<td>4.82</td>
<td>28.43</td>
<td>0.496</td>
<td>2.18</td>
</tr>
<tr>
<td>12</td>
<td>5.00</td>
<td>28.04</td>
<td>0.521</td>
<td>2.18</td>
</tr>
</tbody>
</table>

* D.S. = degree of substitution

C. Replacement of Tosyl Groups by Amino Groups

In order to find the amine which would give the largest amount of replacement of tosyl groups by amine groups, it was decided to prepare a tosylated cellulose which would have a large degree of substitution of tosyl groups. The preliminary amination reactions were run
on a tosylated cellulose with a degree of substitution of tosyl of 1.55. To find an amine which would replace a considerable amount of tosyl was quite difficult. Diethylamine, dibutylamine, and diphenylamine were tried under a large number of reaction conditions, but would not give desirable results. Secondary amines were used to prevent cross linking of the cellulose chains. Finally, the secondary amine pyrrolidine (tetrahydropyrrols) was attempted, and was found to give substantial replacement of tosyl groups even under mild conditions.

It is necessary that the amine which is to be used in the proposed reaction scheme remove all the acetyl groups of tosylated cellulose acetate in addition to replacement of the tosyl groups. When the reaction was attempted with cellulose acetate and pyrrolidine, all the tosyl groups were replaced and all the acetyl groups were removed. The product was isolated by dialysis of the reaction mixture, followed by ice sublimation of the solution to a white fibrous solid. The material was analyzed for nitrogen (4O), sulfur, and acetyl.

(4O) Analysis by Galbraith Microanalytical Laboratories, Knoxville, Tennessee

There was no sulfur, nor any acetyl. The degree of substitution of pyrrolidine can be calculated from the nitrogen analysis in the manner shown previously for tosyl and acetyl. The table below gives the results obtained from a few experiments.
There was apparently complete replacement of tosyl groups by pyrrolidino groups. The small difference in degree of substitution of tosyl and degree of substitution of pyrrolidino groups might well be attributed to analytical errors.

It was shown that pyrrolidine would not react with activated cellulose even after prolonged refluxing. Therefore, it may be assumed that pyrrolidine groups are introduced only when tosyl groups are removed.

This new cellulose derivative has been designated as pyrrolidino cellulose. When prepared by dialysis followed by ice sublimation, the pyrrolidino cellulose is a white fibrous material. It has a large static charge, which makes handling very difficult. It is insoluble in the usual organic solvents, but very readily soluble in

### TABLE II

**SUMMARY OF AMINATION REACTIONS**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>D.S. Tosyl</th>
<th>% Nitrogen</th>
<th>D.S. of pyrrolidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.535</td>
<td>3.73</td>
<td>0.499</td>
</tr>
<tr>
<td>33</td>
<td>0.535</td>
<td>3.62</td>
<td>0.483</td>
</tr>
<tr>
<td>47</td>
<td>0.496</td>
<td>3.80</td>
<td>0.514</td>
</tr>
<tr>
<td>48</td>
<td>0.496</td>
<td>3.55</td>
<td>0.476</td>
</tr>
<tr>
<td>52</td>
<td>0.521</td>
<td>3.61</td>
<td>0.483</td>
</tr>
</tbody>
</table>
water or dilute acid. When a concentrated aqueous solution of pyrrolidino cellulose is poured onto a glass plate, a strong, transparent film results after the water evaporates. Two pieces of hardwood stuck together with an aqueous paste of pyrrolidino cellulose were impossible to pry apart with the bare hands.

D. Hydrolysis of Pyrrolidino Cellulose

The hydrolysis of pyrrolidino cellulose turned out to be a very difficult problem. Later in the work, it was found that in order to completely hydrolyze the polymer, it was necessary to use conditions so drastic that some sugar was decomposed. If some pyrrolidine groups were on carbon 2 of the anhydroglucose unit, then the hydrolysis of that unit would be expected to proceed with difficulty. A similar condition is encountered in the hydrolysis of deacetylated chitin.

E. Chromatographic Separation of the Sugars

The separation of the free sugars was first attempted on a mixture of Florex XXX and Celite (26). The amino sugars did not appear to separate under the conditions used.

Another clay, Florite, was used in the attempted separation of the hexosamine hydrochlorides. Many different developer systems were attempted. The best results were obtained when two V's of absolute methanol were used as the developer. The sugars appeared to separate into three zones besides D-glucose. The D-glucose was eluted through
the column and identified as the phenylosazone. A good deal of difficulty was encountered in isolating the sugars from their zones. They appeared to be so strongly attached that only a small amount could be washed out even when eluting with water. A large amount of inorganic material from the clay was washed out in the process and was very difficult to dispose of. Because of these disadvantages, the isolation of the sugars on clay was considered impractical.

Separation of the sugar acetates on Magnesol and Celite was also attempted (25). Apparently the acetylation of the sugars did not proceed as nicely as would be desired. Poor yields were obtained with acetic anhydride and sodium acetate and with acetic anhydride and pyridine. After many developer systems were attempted, it was found that three V's of 20 - 1 benzene-pyridine gave some separation into zones. The penta-O-acetyl-β-D-glucopyranose was eluted through the column under these conditions and identified by melting point. When the zones were eluted with acetone and then concentrated, nothing but dark amorphous material could be recovered. After much work trying to improve techniques of acetylation and isolation of zones, this method was abandoned.

After the discouraging results obtained from the attempted separation on clay and Magnesol, it was decided to try ion-exchange chromatography. The resin used was Dowex 50 (200 - 400 mesh) (34). Several days were required for the operation of each column.

One zone started coming through the column with the first 100 ml. of developer (.3 N HCl). This zone was identified as D-glu-
cose by paper chromatography. There were also other products in this zone. These were not identified. The second zone did not come through until about 2.7 l. of developer had passed through the column. There were three sharply defined zones besides D-glucose. The fractions from each zone were combined and concentrated to dryness and weighed.

F. Identification of the Sugars

Each zone was compared with glucose by paper chromatography. Zone I was shown to be mostly D-glucose. The $R_g$ values of the components of zones II, III, and IV are 1.13, .76, and 1.21 respectively. The developer was 40-11-19, butanol-ethanol-water. The first zone gave several spots in addition to D-glucose. When the pyrrolidino cellulose was hydrolyzed with refluxing 4 N hydrochloric acid for twelve hours, there was only one spot for zone II, one spot for zone III, and one spot for zone IV. This shows that under these hydrolysis conditions these zones contain only one sugar. When very mild hydrolysis conditions were used to hydrolyze pyrrolidino cellulose, zones II, III, and IV contained very faint spots besides the principal spot shown to be present from stronger hydrolysis experiments.

The sugars could not be obtained in a crystalline form by attempted crystallization from water or alcohol. When the sirups were dried in a drying pistol at 65°C., they could be crushed to a fine glass-like powder. This powder was extremely hygroscopic and would reform into an amorphous sirup after a few minutes contact with the
air. Analysis of Zones II, III, and IV for carbon, hydrogen, and nitrogen gave results which corresponded very closely to the predicted formula $\text{C}_10\text{H}_{20}\text{O}_5\text{Cl}$ for the hexosamine hydrochlorides.

These analyses are given in the experimental section. Analysis of amorphous material is always difficult. The differences between calculated and analyzed values can be attributed to analytical difficulties.

Each of the three zones gave a positive test for chloride ion when the dry sirup was dissolved in water. These facts give strong indication that the sugars are indeed the expected compounds.

It was believed that periodate consumption reactions could be used to determine the position of the pyrrolidine group along the hexose chain. It is known that primary (41) and secondary (42) amines

(41) P. Fleury and A. Guitard, Bull. soc. chim. biol. 28, 651, (1946)
(42) B. Nicolet and L. Shinn, J. Am. Chem. Soc. 61, 1615, (1939)

which are $\alpha$ to an alcohol function will cleave with periodate. These reactions are very rapid. However, Fleury and co-workers (43)


found that tertiary amines $\alpha$ to an alcohol function cleaved very slowly. Triethanolamine consumed only 1.75 moles of periodate after five days. Only .4 moles of ammonia and 2.1 moles of formaldehyde were produced even after ten days. With methyldiethanolamine the re-
action was even slower. Only 0.09 moles of methylamine and 0.21 moles of formaldehyde were produced after three days. The periodate consumption was 1.2 moles. This indicates that periodate is consumed without formation of ammonia and formaldehyde. In the present work, compounds with the following general structure would be encountered:

\[
\begin{align*}
R^* \\
H-C-OH & \quad CH_2-CH_2 \\
H-C-N\cdot HCL & \\
H & \quad CH_2-CH_2
\end{align*}
\]

\[R^* = \text{the remainder of the sugar molecule}\]

This compound would probably be more stable toward periodate cleavage than methyldiethanolamine. At any rate, this group would be cleaved so much slower than adjacent hydroxyl groups that a sharp break in the periodate curve should be observed with the hexosamine hydrochlorides.

When sodium metaperiodate was used as the oxidizing agent, the following results were obtained.

Zone II was found to consume 0.67 moles of periodate rapidly and then slowly consume more. Zone III rapidly consumed three moles of periodate and Zone IV rapidly consumed four moles.
In order to interpret these results, consider the three possible compounds in both the open chain and ring forms. (Page 31).

Upon first consideration, it might be expected that compound (a) would consume three moles of periodate, since (a) should be in equilibrium with (a'). This type of equilibrium is well known in the carbohydrate field. Glucose will take up five moles of periodate readily. However, compound (a) has a tertiary nitrogen in position 2 which would prevent cleavage between carbon atoms 1 and 2, and 2 and 3. Therefore, compound (a) might be expected to consume three moles.

Upon closer inspection, it may be seen that the situation is not as simple as indicated above. The presence of the positive nitrogen atom on carbon 2 could possibly stabilise the ring form to such an extent that the equilibrium is far to the left. This stable hemiacetal would then cleave between carbon atoms 3 and 4. The result of this cleavage would result in the formation of the following compound:

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{HO-} & \quad \text{C-O-} \\
\text{CH}_2-\text{CH}_2 & \quad \text{C-CH}_2\text{OH} \\
\text{CH}_2-\text{CH}_2 & \quad \text{CHO} \\
\text{H} & \quad \text{H} \\
\end{align*}
\]
Open Chain or Ring Forms of the Expected Sugars

(a) 2-deoxy-2-pyrrolidino D-mannose hydrochloride

(b) 3-deoxy-3-pyrrolidino D- allose hydrochloride

(c) 6-deoxy-6-pyrrolidino D-glucose hydrochloride
This hemiacetal would be very stable. The tendency to form

\[ \text{CHO} \quad \text{H} \quad \text{CHO} \]

would be quite small. We would then expect compound (a) to consume one mole of periodate rapidly and then to consume two more moles very slowly.

This reasoning fits the experimental results obtained with Zone II, which was shown to consume less than one mole rapidly and then continue to consume periodate slowly. The latter portion of the curve was linear. When extrapolated to zero time, the curve intersected the ordinate at 0.87 moles. The molecular weight was based on the formula C_{10}H_{20}O_{5}Cl. This agrees well with the expected consumption, considering the possibility that the material may have picked up some moisture during handling. It is also possible that all of the moisture was not removed from the sirup during the drying process. The reaction was followed for eighteen hours and the periodate consumption was still slowly increasing. Therefore, Zone II was considered to be compound (a).

A consideration of equilibrium involved in compound (b) and (b') would indicate that this compound would consume three moles of
periodate. Since this was the amount consumed by Zone III, Zone III was considered to be compound (b).

A similar reasoning showed that compound (c) should consume four moles of periodate. Zone IV, which was shown to consume four moles of periodate, was therefore considered to be compound (c).

The curves for these periodate reactions are shown on page 34. The very small increase in periodate consumption in Zones III and IV after the initial rapid reaction was attributed to overoxidation. The differences in time of completion of the reactions are attributed to differences in concentration of sugar and of periodate.

G. Ratio of the Sugars

The ratio of sugars depends considerably upon the method of hydrolysis. In all cases a very poor yield was obtained. The table on page 35 records some yields of sugars under different hydrolysis conditions.

No conclusive results can be drawn about the quantitative ratio of compounds present. All that could be said was that compound (b) from Zone III is present to a larger extent than compound (a) from Zone II, which, in turn, was present to a larger extent than compound (c) from Zone IV.

From the above results, it may be concluded that in acetone-soluble cellulose acetate the free hydroxyl groups are distributed in an approximately random manner among the 2, 3, and 6 positions of the anhydroglucose unit. The data also indicates that the 3 position predominates in number of free hydroxyls.
TABLE III

RATIO OF SUGARS UNDER DIFFERENT HYDROLYSIS CONDITIONS

<table>
<thead>
<tr>
<th>Column Number</th>
<th>Con. of HCl</th>
<th>Con. of Sugar</th>
<th>Reflux Time</th>
<th>Recovery of Zones</th>
<th>Ratio of Zones</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>4 N</td>
<td>1%</td>
<td>10 hrs.</td>
<td>.0407</td>
<td>.0159</td>
<td>.0160</td>
</tr>
<tr>
<td>17</td>
<td>2 N</td>
<td>4%</td>
<td>17 hrs.</td>
<td>.1125</td>
<td>.0089</td>
<td>.0331</td>
</tr>
<tr>
<td>18</td>
<td>6 N</td>
<td>2%</td>
<td>22 hrs.</td>
<td>.0176</td>
<td>.0058</td>
<td>.0049</td>
</tr>
<tr>
<td>19</td>
<td>1 N</td>
<td>1%</td>
<td>20 hrs.</td>
<td>.1451</td>
<td>.0067</td>
<td>.0284</td>
</tr>
<tr>
<td>20</td>
<td>1 N</td>
<td>1%</td>
<td>24 hrs.</td>
<td>.1288</td>
<td>.0081</td>
<td>.0331</td>
</tr>
<tr>
<td>21</td>
<td>3 N</td>
<td>.5%</td>
<td>10.5 hrs.</td>
<td>.0963</td>
<td>.0142</td>
<td>.0356</td>
</tr>
<tr>
<td>22</td>
<td>3 N</td>
<td>.5%</td>
<td>20 hrs.</td>
<td>.0578</td>
<td>.0113</td>
<td>.0200</td>
</tr>
</tbody>
</table>
V. EXPERIMENTAL

A. Acetyl Analysis

From 200 to 300 mg. of material was carefully weighed and placed in a 500 ml. round bottom flask. The sample was then dissolved in 10 ml. of a 66% aqueous solution of p-toluenesulfonic acid. The rate of solution depended upon the sample. Cellulose acetate usually dissolved completely within four hours, but tosylated cellulose acetate usually required twenty to twenty-four hours. Fifty ml. of distilled water was added to the solution. Usually, a flocculation would occur. This mixture was then placed in the distillation apparatus (39). Steam from the steam line was passed into the flask, which was heated with a low flame from a bunsen burner to keep the liquid level constant. Distillation was continued until 1500 ml. of distillate was collected. The distillation rate was adjusted so that this amount of distillate was collected over a period of six to eight hours. The entire distillate was then titrated with 0.1000 N sodium hydroxide using phenolphthalein as the indicator. A blank was run on the reagents.

B. Sulfur Analysis

Into a Parr bomb was placed 1.0 g. of potassium perchlorate,
15 g. of analytical grade sodium peroxide, .3 g. of benzoic acid, and about .25 g. of weighed sample. The bomb was closed tightly and the contents thoroughly mixed by shaking the bomb. After placing the charged bomb inside the ignition housing, the tip of a Fisher burner was adjusted so it just touched the bottom of the bomb. Water was placed in the appropriate jacket on top of the bomb. After about two minutes an audible ignition occurred. The bomb was quickly quenched in cold water and washed off with distilled water. The bomb was opened and the material adhering to the lid was rinsed into a 600 ml beaker with distilled water. The ignition cup was then placed inside the beaker and 100 ml. of water was added. While the contents of the cup dissolved, a watch glass was placed over the beaker to prevent loss of material. The cup was then rinsed with distilled water.

After neutralization with concentrated hydrochloric acid, the solution was filtered through a sintered glass funnel to remove insoluble material. The filtrate, which was slightly acidic, was transferred to the 600 ml. beaker and warmed to 85 - 90°C. Then 5 ml. of a 10% barium chloride solution was added and the mixture allowed to digest on the steam bath overnight. The barium sulfate was collected and weighed in a porous bottom filter crucible. Quantitative precautions and techniques were observed in all of these operations.
C. Tosylation of Cellulose Acetate

Twenty grams of cellulose acetate (37.95% acetyl; .056 moles of free hydroxyl groups (35)) was dissolved in 120 ml. of pure anhydrous pyridine (44) in a 500 ml. glass stoppered bottle. Solution required twenty-four hours when the mixture was rolled on a mechanical roller. A solution of 14.7 g. (.77 moles) of p-toluenesulfonyl chloride (35) in 240 ml. of pure anhydrous pyridine was added to the above prepared solution of cellulose acetate. The bottle was sealed with sealing wax and mixed on the roller for one hour. The light reddish-tan colored solution was kept at 10°C, for ten days with periodic shaking. Then the solution was poured into 200 ml. of acetone containing enough water (13 ml.) to hydrolyze the excess p-toluenesulfonyl chloride. This reaction was very exothermic and was cooled in an ice bath. The resulting solution was then poured in a very small stream into 6 l. of rapidly stirred iced water. Coagulation of the tosylated cellulose acetate occurred immediately. The fibrous product was filtered through a Büchner funnel as rapidly as possible. Rapid removal of the aqueous solution of by-products probably prevents undesirable side reactions. The product was washed repeatedly with distilled water and then allowed to soak in water for several hours. This process of soaking followed by filtration was continued for two days. After allowing the product to dry in air, it was dissolved in
400 ml. of acetone. This solution was then coagulated into 6 l. of ice water and the product worked up as described previously. After drying in vacuo over phosphorous pentoxide, the yield was 24.1 g.

D. Tosylation of Cellulose

Twenty grams (.375 moles of hydroxyl groups) of cotton linters cellulose (45) was steeped in 20% aqueous sodium hydroxide for forty-five minutes. After removal from the mercerizing bath, the alkali cellulose was placed between stainless steel plates. The excess alkali was removed by placing the plates in a hydraulic press and maintaining a pressure of 5,000 pounds per inch$^2$ for five minutes. This alkali cellulose was then shredded into crumbs by means of a stainless steel rake with sharp teeth. Ageing of the alkali cellulose was accomplished by placing the crumbs in a suction flask equipped with a dropping funnel. The flask was evacuated using the aspirator, and the alkali cellulose was allowed to age for twenty-four hours.

At the end of this ageing period, 12 ml. of carbon disulfide was placed in the dropping funnel and slowly added to the evacuated flask. Cellulose xanthate formed immediately. After standing for one hour, the xanthate was dissolved in 400 ml. of 10% sodium hydroxide. The cellulose was regenerated by coagulating the alkali solution of
xanthate into dilute sulfuric acid. After filtering the regenerated cellulose, washing was continued until the washings were neutral to litmus. The excess water was squeezed out and the damp product placed in a 500 ml. round bottom flask. Pyridine was added and the mixture distilled. In this manner the last traces of water were removed by azeotropic distillation with pyridine at 92.7°C (46). The

(46) M. Le Cat, L'Azeotropisme, Lamertin, 1918, Brussels

distillation was continued until the distillate had the same refractive index as pure pyridine, n

21

D = 1.50919 (47). This procedure gives anhydrous activated cellulose.


The anhydrous regenerated cellulose was dumped into a 500 ml. glass stoppered bottle. A solution of 147 g. (.77 moles) of p-toluene-sulfonyl chloride in 250 ml. of dry pyridine was added. After making the volume up to 360 ml. with more pyridine, the bottle was sealed with sealing wax and mixed on the roller for seven days at room temperature. Complete solution occurred after three or four days. The product was worked up in the same manner as that used for the tosylated cellulose acetate, except that the initial coagulating bath was ethanol. Also, the dried product was not redissolved in acetone and recoagulated. The yield was 53.5 g. of white fibrous material which analyzed for 12.41% sulfur, corresponding to a degree of substitution of tosyl of 1.56.
E. Replacement of Tosyl Groups in Tosylated Cellulose by Secondary Amines

1) With diethylamine

Two grams (0.00776 moles of tosyl) of tosylated cellulose (12.41% sulfur) were placed in a 100 ml. round bottom flask with a ground glass joint. Eighty ml. (.777 moles) of diethylamine (48°) was added and the flask was fitted with a reflux condenser. A potassium hydroxide drying tube was placed in the top of the condenser to exclude moisture. After refluxing for ninety-six hours, the reaction mixture was still heterogeneous. A dark residue was left on the bottom of the flask. The entire reaction mixture was dumped into 500 ml. of ether. A dark insoluble residue was formed which was washed with cold and then with hot water. After drying in vacuo, the yield was 1.45 g. The analysis of 4.1% nitrogen (49°) and 7.13% sulfur corresponds to a degree of substitution of 0.830 diethylamino and 0.699 tosyl groups.
2) With dibutylamine

The above described reaction was attempted with di-n-butylamine (35) instead of diethylamine. The analysis of 4.55% nitrogen (49) and 4.45% sulfur corresponded to a degree of substitution of 1.24 di-n-butylamine and .53 tosyl groups.

3) With diphenylamine

Into a 120 ml. glass stoppered bottle was placed 1 g. (.00388 moles of tosyl) of tosylated cellulose (12.41% sulfur). This was dissolved in 20 ml. of dry pyridine, and 6.3 g. (.037 moles) of diphenylamine (35) was added to the solution. The bottle was tightly stoppered and sealed with sealing wax. The bottle was placed inside an autoclave and water was placed outside the bottle to prevent the lid from coming off when the autoclave was heated. The autoclave was heated to 80°C, for a total of twenty-five hours. After cooling, the reaction mixture was coagulated into ether, and washed with ether and then with water. The yield of dried product was .98 g. The sulfur analysis was 10.41%.

4) With pyrrolidine

Four grams of tosylated cellulose (12.41% sulfur, 1.0156 moles of tosyl) was placed in a 100 ml. round bottom flask. To this was added 40 ml. (.4781 moles) of pure dry
Pyrrolidine (50). Pyrrolidine was dried by allowing it to stand over potassium hydroxide overnight and then distilling it from barium oxide. The tosylated cellulose soon went into solution. The flask was placed in a glycerin bath maintained at 50°C, for a period of fifty-five hours.

Since the other nitrogen-containing cellulose derivatives were insoluble in water, it was assumed that the product of the reaction between tosylated cellulose and pyrrolidine could be obtained by coagulation into water. However, when coagulation was attempted, the resulting product was exceedingly difficult to filter and work up. No better results were obtained upon coagulation into dilute base, or dilute ammonium chloride solution.

In order to obtain a pure product, the entire reaction mixture was dialyzed. The solution was poured into the dialysis bag, which consisted of 28mm. cellulose tubing (51). Water was added to fill the bag six to eight inches below the top. A 850mm. glass tube fitted with a drip tip at the bottom was filled with water. Into this glass tube was placed the dialysis bag and contents. Water was allowed
to flow out the bottom of the apparatus through the drip tip. An 8mm. glass tube was sealed into the neck of a 12 l. round bottom flask. This flask was filled with water and inverted into the water in the outside tube of the dialysis apparatus. As fast as water ran out the drip tip, it was replaced by water from the flask. The large flask required refilling every few hours. Stirring was maintained by means of an air driven stirrer connected to a glass rod which fitted inside the dialysis bag. The glass rod was bent at intervals in order to give good stirring throughout the dialysis bag.

Dialysis was continued for nine days. The product appeared to settle out on the bottom of the dialysis tube when stirring was discontinued. The contents of the dialysis bag were filtered on a sintered glass funnel. After drying the filtered product, the yield was 201g, which contained 4.38% sulfur and 6.8% nitrogen. This corresponded to a degree of substitution of 0.417 tosyl and 1.49 pyrrolidino groups. It is possible that some of the higher nitrogen-containing product may have been sufficiently water-soluble to have been lost during the filtration.

Later it was discovered that a more suitable means of obtaining the product was ice sublimation.
F. Reaction of Activated Cellulose with Pyrrolidine.

An activated cellulose was prepared as described in section D. Five grams of this cellulose was refluxed for seventy-two hours with pyrrolidine. No swelling occurred. The cellulose was filtered and washed repeatedly with water. The analysis gave 0.24% nitrogen (49). Apparently no reaction occurred.

G. Reaction of Tosylated Secondary Cellulose Acetate with Pyrrolidine

Six grams of tosylated cellulose acetate (4.82% sulfur, 26.43% acetyl, tosyl degree of substitution = .496, acetyl degree of substitution = 2.18) was placed in a 100 ml. round bottom flask equipped with a ground glass joint and condenser. Upon addition of 60 ml. (.72 moles) of pure, dry pyrrolidine, the fibrous material dissolved immediately. A potassium hydroxide drying tube was placed at the top of the condenser and the solution in the flask was refluxed for thirty hours. The dark colored solution was cooled and poured into the dialysis bag discussed in section E. Care was taken not to fill the bag too full, because the development of osmotic pressure would cause the contents to overflow. Dialysis was continued for seven days. At the end of this time, the liquid level inside the bag had reached a constant height. The product appeared to be in solution, but a definite Tyndall beam could be observed. So, the product was
very likely in the form of a hydrophilic colloid. The product could not be obtained by boiling off the water either at atmospheric pressure or under reduced pressure, because the colloid foamed quite badly.

A good product could be obtained by ice sublimation. The contents of the dialysis bag were poured into three 1 liter flasks. Each flask contained only about 85 - 90 ml. of liquid. The contents were frozen in a freezing bath in such a manner that the frozen mixture was evenly spread over the inside surface of the flask. Then the ice was sublimed under a vacuum. The yield of white fibrous product was 3.1 g. or 90.6% of theoretical (3.80% nitrogen (40), degree of substitution of pyrrolidino groups = 0.51, 0.00% sulfur, 0.00% acetyl).

Later in the work it was found that a good product could be obtained by coagulating the reaction mixture into absolute methanol. The product was washed with methanol by allowing it to stand in methanol for several hours followed by filtration. This process was continued for two days. The methanol was then replaced by anhydrous ether, and the pyrrolidino cellulose washed for several hours with ether by the process of soaking followed by filtration. After the ether had evaporated, the product was obtained as a yellowish-white powder. Although the nitrogen analysis was the same for the product obtained by this method as that obtained by dialysis and ice sublimation, the yield was slightly lower. Most of the pyrrolidino cellu-
H. Hydrolysis of Pyrrolidino Cellulose

1) With hydrochloric acid

The hydrolysis of pyrrolidino cellulose with hydrochloric acid was attempted using many different reaction conditions. Most of the hydrolysis reactions were carried out in the following manner:

Into a 250 ml. round bottom flask was placed 3.7 g. of pyrrolidino cellulose (3.6 - 3.8% nitrogen). This was dissolved in 200 ml. of 4 N hydrochloric acid. The resulting solution was refluxed for ten hours. During this time the solution became very dark. Most of the color was removed by boiling with charcoal and filtering. This process was repeated three times. The filtrate was then concentrated to a sirup under reduced pressure at 55°C. Excess water was removed by distilling several times with absolute ethanol. The sirup was then placed in a vacuum desiccator over phosphorous pentoxide for several days. This sirup could then be scraped from the sides of the flask in the form of a glass-like powder. The yield was 3.5 g. or 79.4% of theoretical.
2) With sulfuric acid

Into a 500 ml. round bottom flask was placed 3.1 g. of pyrrolidino cellulose and 17 ml. of 72% (52) sulfuric acid. After standing at room temperature for eight days,

(52) G. W. Monier-Williams, J. Chem. Soc., 119, 804 (1921)

the mixture was diluted with 250 ml. of distilled water and refluxed for twenty-four hours. The dark colored solution was decolorized with charcoal. Barium carbonate was added to the filtrate until it was neutral to litmus. Considerable difficulty was encountered in this neutralization. Unless the solution was warmed, neutralization with barium carbonate was very slow. However, if the solution was allowed to become slightly alkaline while it was warm, a red color formed immediately. The same reaction was observed even in the cold, if the alkaline mixture was allowed to stand for some time.

After filtration to remove barium sulfate, the filtrate was concentrated to a sirup under reduced pressure at 55°C. The sugars were extracted with 200 ml. of refluxing methanol. A white insoluble material identified as magnesium sulfate was present in large amounts. This probably resulted from magnesium carbonate impurity in barium carbonate. The hot methanolic solution of sugars was filtered to remove this
inorganic impurity. Concentration of the filtrate was then carried out under reduced pressure. The yield of sirup, after drying as described in part (1), was 3.1 g. or 91.2% of theoretical.

I. Attempted Separation of the Free Sugars on Clay

The free sugars were prepared by hydrolysis with sulfuric acid. The sugar solution was prepared by dissolving 1.0 gram of sirup in 1 ml. of water and 9 ml. of ethanol. In order to avoid useless waste of material, a very small column was used for experimental purposes. This was a tapered pyrex chromatographic column 10 x 130mm (53). The first adsorbent tried was a 5 : 1 mixture of Florex XXX (54) - Celite (55). No advantage was obtained by washing the adsorbent previous to use. However, in order to afford uniform packing, the Florex XXX and the Celite were sifted through an 80 mesh screen.

Considerable care was exercised in packing the column. The adsorbent (3.5 g.) was weighed out and placed in a small beaker. About one-fourth of this was poured through a funnel into the column.

(53) Manufactured by Scientific Glass Company, Bloomfield, New Jersey.


which had a cotton plug on the bottom. Then the aspirator was turned on and the rest of the adsorbent slowly added under aspirator pressure. With the aspirator on, the column was tapped with a wooden dowel, up and down on all sides. The column was now ready for use.

Under aspirator pressure, .5 ml. of the prepared sugar solution was added to the top of the column. An eyedropper was used to wash down the sides of the column. The column was then developed with the prepared developer solution. After all the developer had passed through the column, the aspirator was left on for thirty seconds. The vacuum was then broken suddenly in order to loosen the packing. A cotton plug was placed at the top of the column. The column was then inverted and tapped on a rubber stopper until the packing was loose. Then the column was extruded onto a glass plate. After drying for sixteen hours, the column was streaked with a solution containing 1 part sodium hydroxide, .1 part potassium permanganate and ten parts of water (26). The streak was forced out of a very fine capillary tube by means of an atomizer bulb.

Ten ml. of the following developers were used in the attempted separation:

9 - 1 ethanol - water
3 - 1 ethanol - water
1 - 1 ethanol - water
9 - 1 methanol - water

Under the conditions used, there was no development of the
amine sugars. That the D-glucose was eluted in all cases, was demon-
strated by use of a known sample.

J. Attempted Separation of the Hexosamine Hydrochlorides on Clay

Several columns using D-glucose and D-glucosamine hydrochlor-
ide were run with Florex XXX and Florite (56). The results of these

(56) A clay similar to Florex XXX, manufactured by the Floridin

experiments indicated that Florite gave sharper zones than Florex XXX.
The remainder of the work with clay was therefore carried out using
a 5 - 1 mixture of Florite - Celite which was made to pass through an
80 mesh screen.

The hexosamine hydrochlorides were prepared by hydrolysis in
4 N hydrochloric acid as described in section A. Two grams of this
sirup were taken up in 10 ml. of 7 - 3 methanol - water. The explana-
tory chromatograms were run as described in section I. The follow-
ing developers were attempted:

1 - 1 ethanol - propanol  19 - 1 ethanol - water
2 - 1 ethanol - propanol  9 - 1 ethanol - water
2 - 1 methanol - propanol  4 - 1 ethanol - water
2 - 1 methanol - ethanol   100 - 1 methanol - water
1 - 1 methanol - propanol  10 - 1 methanol - water
      ethanol              5 - 1 methanol - water
      methanol            1 - 1 methanol - water
                        1 - 2 methanol - water
                        water
Each column was run with different amounts of each developer solution. Usually 1, 2, 3, and 4 V's of each developer were attempted. A total of 101 exploratory chromatograms were attempted on Florite using the hexosamine hydrochlorides. The chromatographic procedure was the same as that described previously.

It was found that 10 cc. (2 V's of methanol gave three fairly well defined zones besides D-glucose, which was eluted through the column under these conditions. The three zones were at 0 - 20mm., 25 - 35mm., and 50 - 70mm. The distance was measured from the top of the column of adsorbent.

This technique was then applied to a column 35 x 230mm. Eighty grams of the adsorbent (Florite - Celite, 5 - 1) was carefully packed into the column. After prewashing the column with 160 ml. of methanol, 10 ml. of the prepared sample solution was added to the top of the column. The column was then developed with 230 ml. (2 V's) of methanol. Location of the zones was accomplished in the same manner as described previously. The three zones were detected at 0 - 40mm., 50 - 70mm., and 110 - 150mm.
Following the removal of the streak, each zone was cut out and repacked into a column. Then 150 ml. of water was used to elute the sugars. Even after this treatment, the adsorbent still retained some reducing components. The effluent from each elution was concentrated to dryness at 55°C. under reduced pressure. Twenty ml. of water was added and the flocculated adsorbent removed by filtration. The filtrate was again concentrated to dryness, and 20 ml. of water added. Once more the flocculated adsorbent was removed by filtration and the filtrate concentrated to a small volume. When ethanol was added, more flocculation occurred. The flocculated material was filtered and the process repeated two more times. A large amount of ethanol was then added and the solution placed in the refrigerator over night. White crystals, which did not melt below 290°C., were formed. Qualitative analysis showed that these crystals were sodium chloride.

The above technique was attempted with D-glucosamine hydrochloride. The zone appeared at the top of the column. After elution to remove the sugar, the same method was attempted to isolate the D-glucosamine hydrochloride. Again the crystals which formed were sodium chloride.

In another column using the hydrolysis mixture, the zones were cut out and eluted in the usual manner. After the usual concentrations, additions of water, and filtration to remove flocculated adsorbent, the product was taken up in a few ml. of water. Then .8 g.
of phenylhydrazine hydrochloride and 1.2 g. of crystalline sodium acetate were added and the solution heated in a boiling water bath for fifteen minutes. No visible precipitate formed upon cooling.

A third method was attempted to isolate the sugars. The eluted zones were treated in the usual fashion to remove flocculated adsorbent and then concentrated to dryness. Then, .4 g. of freshly fused sodium acetate was heated with 5 ml. of acetic anhydride. The boiling mixture was poured into the flask containing the dried eluted zone. After boiling for five minutes, the mixture was poured into 50 ml. of ice water which was stirred rapidly. No precipitate formed.

Another method used to attempt the isolation of the zones was to remove flocculated material and inorganic material in the usual fashion and then allow the solvent (water and ethanol) to evaporate off in crystallizing dishes. The solvent evaporated after a few hours, but no crystals were formed even after several days.

The effluents which came through the above columns, when developed with 2 V's of methanol, were treated in the following manner:

The effluent from one column was heated with charcoal and filtered and concentrated to a small volume. The material was washed into a test tube containing 2.0 g. of phenylhydrazine hydrochloride and 3.0 g. of crystalline sodium acetate in 15 ml. of water. After heating in a boiling water bath for fifteen minutes, the reaction was cooled and filtered. The osazone was recrystallized from alcohol and dried.
K. Preparation of the Sugar Acetates

1) Acetylation with acetic anhydride and pyridine

The sirup was obtained by hydrolysis with sulfuric acid in the manner described in section H. Into a flask containing 3.1 g. of dried sirup was poured 40 ml. of acetic anhydride and 40 ml. of dry pyridine. After vigorous shaking for one hour, the mixture was allowed to stand for twenty-one hours at 10°C. with periodic shaking. The mixture was then shaken at room temperature for two hours and the insoluble material removed by filtration. The filtrate was concentrated to a sirup under reduced pressure at 55°C. This sirup was shaken with a saturated solution of sodium bicarbonate to neutralize any acid present and to liberate the free amine. Three 75 ml. portions of chloroform were used to extract the acetylated sugars. The chloroform layer was dried over anhydrous sodium sulfate and concentrated to a dark colored sirup under reduced pressure. The yield was 4.0 g. or 66.4% of the theoretical.

2) Acetylation with acetic anhydride and sodium acetate

The material for acetylation was prepared by hydrolysis with sulfuric acid. After drying in a vacuum desiccator
for several days over phosphorous pentoxide, the sirup (5.7 g.) was crushed into a powdered form. In a 125 ml. Erlenmeyer flask 40 ml. of acetic anhydride was boiled with 3 g. of freshly fused sodium acetate. When the powdered sirup was slowly added to this mixture, a self-sustaining reaction ensued. After the reaction had subsided, the flask was cooled and the contents poured into 300 ml. of ice water which was rapidly stirred. A brown flocculation occurred which soon settled out. After the solution was made basic by addition of solid sodium bicarbonate, the acetylated sugars were extracted with chloroform and the product worked up as described previously.

The yield of dark colored sirup was 5.0 g. or 44.0% of the theoretical.

In the acetylation with either acetic anhydride and pyridine or acetic anhydride and sodium acetate, there appeared to be considerable decomposition. It was believed that this may have been due to the instability of the free amino sugars. The acetylation reactions were carried out again using the hexosamine hydrochlorides. No better results were obtained.
L. Attempted Separation of the Sugar Acetates on Magnesol (57)

(57) Manufactured by the Westvaco Chlorine Products Corp., South Charleston, West Virginia.

The adsorbent used in this attempt was a 5 - 1 mixture of Magnesol - Celite (25). Both components were sifted through an 80 mesh screen before mixing. The sample solution was prepared by dissolving 5.0 g. of the acetylated sugars in 20 ml. of benzene.

Exploratory chromatograms were run in the following manner:

The column was carefully packed with 2.7 g. of the adsorbent mixture. Then under full aspirator pressure, .2 ml. of sample solution mixed with .3 ml. of benzene was added to the top of the column. Development of the column and detection of zones were accomplished in the same manner as with the clay column, except that the drying period previous to streaking was omitted. The various developers used were:

100 - 1 benzene - t - butyl alcohol
50 - 1 benzene - t - butyl alcohol
50 - 1 benzene - pyridine
30 - 1 benzene - pyridine
20 - 1 benzene - pyridine
30 - 1 benzene - acetic acid

Many different volumes of these developer systems were attempted. A total of thirty-nine exploratory chromatograms were run. The
sharpest zones appeared when 3 V's of 20-1 benzene-pyridine was used. Under these conditions, penta-O-acetyl-\(\beta\) -D-glucopyranose was eluted through the column. Three sharp zones remained on the column at 0 - 10mm., 27 - 30mm., and 47 - 65mm.

Chromatography of the sugar acetates was then attempted on a larger scale. Sixty-five grams of adsorbent was packed in a larger column (35 x 230mm.) and 20 ml. of the sample solution was added to the top of the column. After development with 360 ml. (3 V's) of 20-1 benzene-pyridine, the column was extruded and streaked in the usual manner. Sharp zones were located at 0 - 20mm., 45 - 55mm., and 90 - 110mm. Each zone was cut out and made into a slurry with acetone. The slurries were filtered through a sintered glass funnel, and the filtrates concentrated to dark colored sirups under reduced pressure at 55°C. Each fraction was taken up with 95% ethanol.

Zones from five columns were combined in this manner and placed in crystallizing dishes. An amorphous sirup, which did not crystallize, was formed from each zone.

These sirups were then taken up in a small amount of hot 95% ethanol. Hot water was added to each solution until the cloud point was almost reached. After standing at 10°C. over night, only a small amount of highly colored amorphous product was obtained. These were discarded.

The effluents from the above columns were combined and concentrated to a sirup. Rechromatography of this sirup using 3 V's of
30 - l benzene-acetic acid showed four more zones. Penta-O-acetyl-\(\beta\) -D-glucopyranose was eluted through the column. Again, it was impossible to crystallize the material eluted from the zones.

The effluent from this latter chromatographic procedure was concentrated to a sirup which soon crystallized. After three recrystallizations from 75% ethanol, the crystalline product had a melting point of 131 - 131.1 C. The literature value for the melting point of penta-O-acetyl-\(\beta\) -D-glucopyranose is 131 C. (58).


M. Separation of the Sugars on an Ion Exchange Resin.

1) Preparation of the column

The ion exchange resin used in this work was Dowex 50 (59) (200 - 400 mesh) (34). Before each chromatographic separation the resin was cleaned and recharged in the following manner:

Enough of the resin to fill the column was made into a slurry with distilled water. The slurry was poured into a sintered glass funnel. Under full aspirator pressure, l
liter of 10% sodium hydroxide was passed through the resin. This was followed by 1 liter of distilled water. Then 10% hydrochloric acid was passed through the resin until the filtrate showed no flame test for sodium ions. After an additional 500 ml. of .3 N hydrochloric acid had passed through the resin, a slurry was made of the resin with this acid. The chromatographic column was made by sealing a piece of 6mm. glass tubing onto a piece of glass tubing 25 x 540mm. A piece of Tygon tubing was placed on the 6mm. tube and closed by means of a screw clamp. A porcelain plate from a Gooch crucible was placed in the bottom of the column. On top of this, a wad of glass wool was carefully placed. About 3 inches of .3 N hydrochloric acid solution was poured into the column. The slurry of resin was very carefully poured into the prepared column until the column was full. After a few minutes the screw clamp was released. As the level of resin fell, it was replaced by more slurry. Resin was continuously added until the level remained about 50mm. from the top of the column.

A tight fitting rubber stopper with a 6mm. glass tube through the middle was placed in the top of the column, and one end of a piece of Tygon tubing was connected to the glass tube. The other end of the piece of Tygon tubing was connected to the stem of a 500 ml. separatory funnel.
separatory funnel was filled with 0.3 N hydrochloric acid, and raised above the top of the column. The drop rate from the column was regulated by the height of the separatory funnel from the top of the column. When the pH of the effluent was equal to the pH of the acid passing into the resin, the column was equilibrated and ready for use.

Operation of the column

Several different hydrolysis conditions using hydrochloric acid were used to obtain the sugars for use in this column. These are summarized in the discussion of experimental results. In general, the hydrolysis was similar to the method described in section H.

One-half gram of sirup was dissolved in 10 ml. of 0.3 N hydrochloric acid. A porcelain plate from a Gooch crucible was placed at the top of the column of resin to prevent agitation. The solution of the sample was added slowly. In order to prevent trailing of zones, the following technique was carried out:

The sample solution was allowed to pass completely into the resin. This process was very slow unless pressure was applied to the top of the column. An atomizer bulb was attached to a piece of Tygon tubing which was connected to a glass tube in a rubber stopper. When the stopper was fitted into the column, the atomizer bulb was compressed.
Unless the stopper was held firmly in the column with one hand, the pressure generated by the atomizer bulb would force it out. When all of the sample solution had passed into the resin, the sides of the column were washed down with 0.3 N hydrochloric acid. This was also forced through the column by using pressure. Then eight or nine 4 ml. portions of 0.3 N hydrochloric acid were forced down the column in this manner. Extreme care was observed to prevent the column from becoming dry. This procedure enabled the sugars to be developed down the resin before the developer was added.

In all columns, 0.3 N hydrochloric acid was used as the developer. The addition of the developer was carried out using the same procedure as described in the equilibration process. In order to obtain a reasonable flow of developer, the separatory funnel was raised three or four feet above the top of the column. Even with this amount of hydrostatic pressure, a maximum of 25 drops or 2.3 ml. per minute was obtained. A constant pressure head was maintained by use of a stoppered 2 l. separatory funnel placed above the first one.

Fractions were taken by means of an automatic fraction collector (60). A timer could be set at any interval. This enabled the collection of fractions of any desired size. Usually, the fractions were between 50 - 60 ml. when collect-
ing effluent between the first and second zone. When the zones were closer together as in Zones II, III, and IV, the time was set to collect fractions from 30 - 40 ml.

3) Detection of the reducing zones

A very simple procedure was developed which would detect even trace amounts of sugar. Three drops from each fraction were placed on a spot plate. Two drops of 10% sodium hydroxide solution and two drops of alkaline potassium permanganate were added to each spot. The alkaline potassium permanganate solution was prepared by dissolving .1 g. of potassium permanganate in 100 ml. of 10% sodium hydroxide. Spots which contained sugars immediately turned from purple to green.

A reducing zone started coming through the column after 60 ml. of developer had passed through. This zone extended over a volume of 200 ml. A second reducing zone came through from 2,500 to 2,725 ml., a third zone from 3,000 to 3,300 ml., and a fourth from 3,800 to 4,050 ml. No more zones were eluted upon allowing 1500 ml. of developer to pass through the column after Zone IV was collected.

4) Isolation of zones

All the fractions of a zone were combined and concentrated to a small volume under aspirator pressure at 55°C. or lower. The concentrated zone was transferred to a 10 ml.
round bottom flask. The solution was then concentrated to a sirup, and the flask containing the sirup was placed in a drying pistol at 65°C for several hours. After weighing the flask and its contents very accurately, the sirup was removed from the flask in the form of a glass-like powder. The flask was then washed out with water, dried, and reweighed. In this manner a quantitative determination of the amount of sugar in each zone was obtained. These results are discussed in the section on discussion of experimental results.

N. Identification of the Zones from the Separation on Dowex 50

1) Analysis

Analysis was carried out on the powdered sirup prepared as described previously. No analysis was carried out for Zone I since this zone consisted mostly of D-glucose.

It was believed that the pyrrolidino sugars should exist as the pyrrolidine hydrochlorides. To verify this, a small amount of sirup from each zone was dissolved in a few ml. of water and acidified with nitric acid. When silver nitrate solution was added, Zones II, III, and IV gave a white precipitate soluble in ammonium hydroxide. A similar reaction was observed with D-glucosamine hydrochloride. If the compounds were present as the hydrochlorides of the tertiary nitrogen, then the analysis would have been 5.18% N,
The analysis found was: (40)

Zone II 4.77% N, 7.70% H, and 42.57% C
Zone III 5.25% N, 7.77% H, and 44.10% C
Zone IV 5.23% N, 7.74% H, and 43.27% C

2) Attempted crystallization

Zones II, III and IV were dissolved in hot ethanol. Hot ethyl acetate was added until the solutions turned cloudy. The solutions were then cleared up by adding just enough ethanol to remove the cloudiness. Upon standing for several hours at room temperature and several more at -10°C, no crystals were formed in any of the flasks. A small amount of white amorphous material was present in each. When acetone, ethyl ether, or petroleum ether was used as the precipitating agents, the same results were obtained.

The sugars could not be made to crystallize by cooling a hot, concentrated, alcoholic solution.

If a large excess of acetone or some other precipitating agent was added to the ethanolic solution, a considerable amount of white amorphous material formed immediately. Upon attempted filtration, this amorphous material formed a sirup.

An attempt was also made to crystallize the sugars from an aqueous solution at 50°C. The solutions were placed
on watch glasses and placed in an oven at 50°C. No crystals formed even after several days.

An attempt was made to prepare the picrates of the amine sugars. When concentrated ethanolic solutions of the hexosamine hydrochlorides were added to concentrated solutions of picric acid in ethanol, nothing except picric acid crystallized upon cooling.

3) Paper chromatography of the sugars

In order to determine the purity of each zone, paper chromatograms were run. A few mg. of each zone were dissolved in 1 ml. of water and a spot placed on the paper chromatograms. D-glucose was used as a reference. A mixture of butanol - ethanol - water (40 - 11 - 19) was used to develop the chromatograms. After twelve to eighteen hours, the papers were removed from the developer and allowed to dry in air. Then a 2% weight-volume solution of p-anisidine hydrochloride in butanol was sprayed onto the papers until the entire surfaces were wet. After allowing the papers to dry in air, they were placed in an oven saturated with vapor at 105°C. for ten minutes. The sugar spots were dark brown on a tan background. Very faint spots were detected by their fluorescence under ultraviolet light.

Zone I had a large spot which corresponded to D-glucose. In addition, there were other spots which were quite faint but distinguishable.
Zones II, III and IV showed only one spot when the pyrrolidino cellulose was hydrolyzed with refluxing 4 N hydrochloric acid for twelve hours. When refluxing 1 N hydrochloric acid was used to hydrolyze the polymer, Zones II, III, and IV were not pure. Even after refluxing for twenty-four hours with this acid there were faint spots besides the main sugar spots. The minimum conditions required to give only one spot on paper, were hydrolysis of the polymer with refluxing 3 N hydrochloric acid for ten to eleven hours.

The chromatograms shown on page 68 are from zones obtained by hydrolysis with refluxing 4 N hydrochloric acid for ten to eleven hours.

The \( R_G \) (61) values of the compounds obtained from

\[
(61) \quad R_G \text{ value} = \frac{\text{distance moved by sugar}}{\text{distance moved by D-glucose}}
\]

Zone II, III and IV are 1.13, .75, and 1.21.

4) Periodate analysis

The periodate consumption was determined for every zone, except Zone I which was shown to be mostly D-glucose.

Periodate analysis of Zone II was carried out in the following manner:

The amount of sugar present was determined by weighing the 10 ml. round bottom flask containing the dried sirup. Five ml. of water was then added to the flask to dissolve the
PAPER CHROMATOGRAMS

Zone I

O       O       O       O

X       X

D-glucose

Zone II

O

X

Zone III

X

L-glucose

Zone IV

O

X

L-glucose

Chromatograms shown are one-half actual size.
sugar. This solution was poured into a 125 ml. Erlenmeyer flask and allowed to drain thoroughly. Then 5 ml. of .0625 M sodium metaperiodate were added to the 10 ml. round bottom flask. Again this was drained into the 125 ml. Erlenmeyer flask. The Erlenmeyer flask was then stoppered. The round bottom flask was dried and reweighed. The weight of sugar was .0115 g. At intervals 1 ml. of the periodate reaction mixture was pipetted into 25 ml. of .00417 N sodium arsenite solution containing a large amount of solid sodium bicarbonate and 2 ml. of ½ M potassium iodide solution. After standing for fifteen minutes, the excess arsenite was titrated with .00417 N iodine solution using starch as an indicator.

To obtain the amount of periodate consumed, a blank was run on the reagents. Also, the iodine was standardized against the sodium arsenite solution. By doing this, the calculation may be simplified to the following formula:

\[
\frac{\text{moles of sugar}}{\text{moles of periodate}} \times \text{ml. of arsenite consumed in blank} = \text{ml. of arsenite corresponding to 1 mole of periodate consumed per mole of compound.}
\]

This method cancels out most experimental errors, and errors due to incorrect concentration of iodine and arsenite.

The above procedure works nicely for quantities of sugar ranging from five to twenty mg. This procedure was
worked out mostly for periodate analysis on Zone II, which was found to consume one mole rapidly and then slowly consume more. Although this procedure was used successfully for small amounts of Zones III and IV, a different procedure was used to obtain the periodate curves of these zones reproduced on page 34.

For Zone IV, 0.0749 g. of sugar was dissolved in 5 ml. of water and 5 ml. of .2502 M sodium metaperiodate. Two ml. aliquots were pipetted into 25 ml. of .0252 N sodium arsenite containing a large excess of solid sodium bicarbonate and 2 ml. of 4 M potassium iodide solution. After standing for fifteen minutes, the excess arsenite was titrated with .0252 N iodine solution.

The periodate curve for the analysis of Zone III was obtained on .0319 g. of sample using .2502 M periodate and .0252 N arsenite and iodine.

At least two curves were obtained on each zone. The results always came out the same for the same zone. This indicates that the periodate analysis was accurate.

5) Specific rotation of the sugars

In order to more fully characterize the new sugars, specific rotations were taken. Accurate weighings of the amount of sugar present were obtained in the manner described for periodate analysis. After the flask and contents were
accurately weighed, 5 ml. of water were added to the flask. After the sugar had completely dissolved, a two decimeter semimicro polarimeter tube was filled with the solution. The rotation of the slightly colored solution was taken. A zero reading was obtained on the polarimeter tube filled with water.

Zone II had a constant rotation of $+47.8^\circ$ at $27^\circ$C.

Zone III appeared to mutarotate from $+55.40^\circ$ to $+41.3^\circ$ at $25^\circ$C. after twenty-four hours.

Zone IV mutarotated from $-18.7^\circ$ to $-9.76^\circ$ at $25^\circ$C. after twenty-four hours.

The D line of sodium was used in taking all readings.
VI. SUMMARY AND CONCLUSIONS

In the course of this work, a new cellulose derivative, pyrrolidino cellulose, was prepared. The hydrolysis of this cellulose derivative with hydrochloric acid yielded three new amino sugars. These were:

2-deoxy-2-pyrrolidino-D-mannose hydrochloride;
\[ [\alpha]_D^{27} = +17.8 \text{ (water)}, \]

3-deoxy-3-pyrrolidino-D-allose hydrochloride;
\[ [\alpha]_D^{25} = +11.3 \text{ (water)}, \text{ and} \]

6-deoxy-6-pyrrolidino-D-glucose hydrochloride;
\[ [\alpha]_D^{25} = -9.76 \text{ (water)}. \]

This work has shown conclusively that the position of the free hydroxyl groups in acetone-soluble cellulose acetate is not limited to any one position. It was shown that these free hydroxyl groups are distributed among the 2, 3, and 6 positions of the anhydroglucose unit. The available data indicated that the number of free hydroxyl groups in the 3 position is greater than that in the 2 position, which in turn is greater than that in the 6 position.
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AUTOBIOGRAPHY

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