Sonderdruck

aus der Fachzeitschrift „Die Stärke“

Jahrgang 17, Heft 6, Seite 176–179

1965
Location of Xanthate Groups in Starch Xanthate

By W. M. Doane, C. R. Russell, and C. E. Rist, Peoria, Illinois (USA)

Introduction

Starch xanthates prepared by a rapid, continuous process were previously shown to be uniformly substituted (1). Virtually no unreacted starch was present, and the amylose and amyllopectin components were substituted to the same degree. Analysis of the amylose and amyllopectin components was preceded by quantitative replacement of the xanthate group with a methyl group by a sequence of reactions previously applied to cellulose xanthates (2). The procedure entails stabilization of the xanthate group by reaction with benzyl bromide and then benzoylating to block all free hydroxyls. The S-benzyl xanthate group is removed with mercuric acetate and hydrogen peroxide, and the product methylated and debenzoylated to yield the O-methyl starch. Starch xanthates of degree of substitution (D.S.) 0.12 and 0.33 were converted to O-methyl starches of D.S. 0.13 and 0.32, respectively. These products were then examined by quantitative chromatographic procedures to determine the distribution of O-methyl groups among the 2-, 3-, and 6- positions. The values obtained represent the actual distribution of xanthate groups in the original starch xanthates.

Numerous studies on the location of xanthate groups in cellulose xanthate have been made, but only ADAMEK and Purves (3) have published on the location of the xanthate substituents in starch xanthates. They used tritylation techniques to determine the amount of primary xanthate substitution. After 100 minutes of xanthation, they found 38% of the xanthate groups on the primary position; after 3,330 minutes of xanthation, 36% of the remaining xanthate groups occupied the primary position in the starch molecule. Prelimi-
nary experiments with our continuous xanthation products have also indicated an increase in substitution at the primary position with time. This increase with time in the substitution at C-6 is being studied in more detail.

**Experimental**

**Xanthation**

Starch was converted to starch sodium xanthate at desired levels of substitution by a continuous xanthation process (4). By reacting commercial corn starch, aqueous sodium hydroxide, and carbon disulfide in molar ratios of 1:1:1/2 and 1:1:2:1:6, respective xanthates of D.S. 0.33 and 0.12 were prepared. In both preparations the carbon disulfide was introduced before the sodium hydroxide solution was. After a residence time of 2 minutes in the mixer-reactor, the highly viscous reaction masses were discharged and allowed to stand for 1 hour at 25°C before dissolution to form 10% aqueous stock solutions. Portions of these solutions were immediately removed for reaction with benzyl bromide.

**Replacement of Xanthate Group with a Methyl Group**

The quantitative replacement of the xanthate group with a methyl group in starch xanthates of D.S. 0.12 and 0.33 was carried out as previously reported (1) and gave O-methyl starches of D.S. 0.13 and 0.32, respectively, with overall recoveries of approximately 70%.  

**Location of O-Methyl Groups**

**Hydrolysis.** The O-methyl starches (0.5 g) were allowed to stand overnight in 5 ml of 72% sulfuric acid. Water was then added to reduce the acidity to approximately 1 N and the solutions were refluxed for 4 hours. After hydrolysis, the solutions were neutralized with barium carbonate and the barium sulfate was removed by filtration. The hydrolyzates were diluted to exactly 300 ml, and the total amount of reducing sugar was determined by the iodometric micro-procedure described by Hirst, Hough, and Jones (5).

**Quantitative Paper Chromatography.** The hydrolyzates were concentrated to small volumes and streaked quantitatively on Whatman No. 1 filter paper cut in 9 x 22 inch strips and sarrated at the lower edge. The top layer of a 4:1:5 mixture of n-butanol:ethanol:water (6) was allowed to flow down the paper for 24 hours to separate the hydrolyzate components. Appropriate guide strips were cut from the chromatograms and sprayed with a 3% solution of p-anisidine hydrochloride in l-butanol. The sugar spots appeared after heating the guide strips at 100°C for 15 minutes in an oven. Comparison of the Rf values with those of authentic samples indicated the presence of D-glucose, and both mono- and di-O-methyl-D-glucose in the two samples. After the guide strips were repositioned, appropriate areas of the chromatograms corresponding to the developed areas of the guide strips were removed and cut into small pieces approximately 1-cm square. The sugar component was eluted from the excised areas by repeated washings with hot water. Concentra-

Following determination of the amount of reducing sugar in each of the three components, the mono-O-

methyl sugars were further separated following the chromatographic procedure of Lenz and Holmberg (7) with the top layer of a 2:5:5 mixture of 2,4,6-collidine:ethyl acetate:water as irrigant. Comparison of excised guide strips with chromatograms containing authentic samples of 2, 3, and 6-O-methyl-D-glucose identified these components when present. The amount of each of the sugars in the remaining portions of the chromatograms was determined quantitatively as before. These values, when corrected for the amount of sugar in the guide strips, showed nearly theoretical recovery.

Further separation of the di-O-methyl-D-glucose component from the higher D.S. product was attempted by rechromatographing the mixture and allowing the same solvent, as used in separating the mono-O-methyl sugars, to pass down the chromatogram for 16 hours. The chromatograms were removed every 4 hours and air dried in a hood in an attempt to sharpen the zones occupied by the various components. Separation was not so complete as with the mono-O-methyl components, but comparison of the developed chromatograms with known samples of 2,3, and 2,6-di-O-methyl-D-glucose identified these components.

**Tritylation**

Tritylation of the D.S. 0.33 S-benzyl xanthate was performed by using a 4-mole excess of triphenylmethyl chloride in anhydrous pyridine at 50°-60°C for 72 hours. Attempts to use higher temperatures and shorter reaction times occasioned losses in sulfur content. Following tritylation, the reaction solution was transferred quantitatively to a blender containing absolute ethanol; the resulting precipitate was collected on a tared, fritted glass funnel and washed thoroughly with ethanol then finally with ether before drying to constant weight. The degree of tritylation was calculated from the total weight of the isolated product and agreed with the value calculated from the sulfur content of the product.

**Results and Discussion**

Starch xanthates of D.S. 0.12 and 0.33 were converted to the corresponding O-methyl starches of D.S. 0.13 and 0.32. Chromatographic examination of the O-methyl starch hydrolyzates revealed three components in each. Comparison of the chromatograms with authentic samples of D-glucose and its mono- and di-O-methyl ethers identified the three substituents. Quantitative determination of the sugars after elution from the chromatograms gave the results summarized in Table 1. Total recovery of chromatographed hydro-

<table>
<thead>
<tr>
<th>Sugar</th>
<th>D. S. 0.32</th>
<th>D. S. 0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of Total</td>
<td>% of Total</td>
</tr>
<tr>
<td></td>
<td>Recovered</td>
<td>Recovered</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>328</td>
<td>406</td>
</tr>
</tbody>
</table>
| Mono-O-methyl   | 150        | 30.0       | 54.1   | 11.7
| Di-O-methyl     | 12.2       | 2.3        | 1.4    | 0.3    |

1) 497 mg chromatographed.
2) 470 mg chromatographed.

<table>
<thead>
<tr>
<th>Table 1 Composition of O-Methyl Starch Hydrolyzates</th>
<th>D. S. 0.32</th>
<th>D. S. 0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>% of Total</td>
<td>% of Total</td>
</tr>
<tr>
<td></td>
<td>Recovered</td>
<td>Recovered</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>328</td>
<td>406</td>
</tr>
</tbody>
</table>
| Mono-O-methyl                                    | 150        | 30.0       | 54.1   | 11.7
| Di-O-methyl                                      | 12.2       | 2.3        | 1.4    | 0.3    |

1) 497 mg chromatographed.
2) 470 mg chromatographed.
lyzate was nearly theoretical in each case. The mole ratios of d-glucose:mono-O-methyl-d-glucose:di-O-methyl-d-glucose were 32:13:1 and 34:4:0.1 for the respective D.S. 0.32 and 0.13 products.

The mono-O-methyl fractions were further resolved chromatographically, and the developed areas of the chromatograms were compared to authentic samples of 2-, 3-, and 6-O-methyl-d-glucose. The D.S. 0.32 mono-O-methyl fraction contained all three O-methyl ethers while the lower D.S. hydrolyzate contained only the 2- and 6-O-methyl ethers. The isomer present in greatest abundance in both hydrolyzates was 6-O-methyl-d-glucose as shown in Table 2. It accounted for 67\% of the mono-O-methyl components in the D.S. 0.32 starch and 56\% in the D.S. 0.13. This distribution indicates that the primary position is the most readily xanthated in starch under the conditions employed. The hydroxyl at C-2 was somewhat less reactive accounting for 27\% of the higher and 44\% of the lower D.S. mono-O-methyl components. The hydroxyl at C-3 appears rather inactive towards xanthation. At the xanthation level of 0.12 none of the xanthate groups were on C-3. At a D.S. level of 0.33 where about 31 anhydro-d-glucose units in 100 contained a single xanthate group less than 2 d-glucose units were xanthated at C-3. This position has also been shown to be the least reactive towards xanthation in cellulose (2).

The di-O-methyl fraction from the D.S. 0.32 product was rechromatographed in attempts to identify its components. Comparison of the chromatograms with authentic samples of 2,3- and 2,6-di-O-methyl-d-glucose identified both of these sugars. The latter isomer appeared to be in greatest abundance although no quantitative determination was made. The intensity of the area occupied by the 2,3-isomer suggested that it was present in only trace amounts. A sample of 3,6-di-O-methyl-d-glucose was not available for chromatographic comparison.

Tritylation of the D.S. 0.33 S-benzyl xanthate was performed for comparison of the amounts of primary substitution determined by this method with that of the chromatographic procedure employed. The xanthate product was tritylated to a D.S. of 0.76 and could not be increased by repeated tritylations. The difference between unity and 0.76 indicates that 0.24, or 76\%, of the xanthate groups occupied the primary position. Assuming that the di-O-methyl-d-glucose fraction consists mostly of 2,6- or 3,6-di-O-methyl-d-glucose, or both, a D.S. of 0.23 is calculated for the primary xanthate substitution as found chromatographically. The close agreement of the values obtained by the two methods indicates that no benzoyl migration occurred during the dehydroxylation and methylation reactions.

Acknowledgement

We are grateful to CLARA McGREW and BONITA HEATON for methylxanthate determinations and to JOHN HOGG and JOHN VAN CLEVE for supplying samples of some of the D-glucose-O-methyl ethers.

Summary

The distribution of xanthate groups among the 2-, 3-, and 6-positions of starch xanthates prepared by a rapid, continuous process is reported. Starch xanthates of degree of substitution (D.S.) 0.12 and 0.33, prepared in equal reaction time, were stabilized by reaction with benzyl bromide. Complete benzoylation of the starch S-benzyl xanthate, followed by removal of the xanthate groups with mercuric acetate and hydrogen peroxide, permitted the isolation of a starch benzoate which, on subsequent methylation and debenzylation, yielded O-methyl starches possessing a methyl group at every position previously occupied by a xanthate group.

Chromatographic analysis of the products after acid hydrolysis of the O-methyl starches showed that d-glucose and its mono- and di-O-methyl ethers were present in mole ratios of 34:4:0.1 and 32:13:1 for the lower and higher D.S. products, respectively. Resolution of the 0.32 O-methyl component of the D.S. 0.33 product showed 67\% 6-, 27\% 2-, and 6\% 3-O-methyl-d-glucose. The lower D.S. product showed 56\% 6- and 44\% 2-O-methyl-d-glucose but no 3-O-methyl-d-glucose. Tritylation of the D.S. 0.33 S-benzyl xanthate confirmed the amount of primary xanthate substitution as found chromatographically.

Zusammenfassung


Table 2

<table>
<thead>
<tr>
<th>Mono-O-Methyl D-Glucose</th>
<th>D.S. 0.32</th>
<th>D.S. 0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg Found% of Total Recovered</td>
<td>Mg Found% of Total Recovered</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>37.8</td>
<td>27</td>
</tr>
<tr>
<td>3-</td>
<td>8.4</td>
<td>6</td>
</tr>
<tr>
<td>6-</td>
<td>94.0</td>
<td>67</td>
</tr>
</tbody>
</table>

1) 144 mg chromatographed.
2) 48 mg chromatographed.
On donne un compte rendu de la distribution des groupes xanthates sur les positions 2-, 3- et 6-, des xanthates d'amidon. Ces xanthates ont été préparés par un procédé continu rapide. Des xanthates d'amidon ayant un degré de substitution (D. D.) de 0,12 et 0,33 ont été préparés dans le même temps de réaction, ont été stabilisés par réaction avec bromure benzyle. La benzoylation complète du xanthate S-benzyle de l'amidon suivie de l'enlèvement des groupes xanthates a conduit à l'isolement et un benzoate d'amidon qui après méthylation et débenzoylation, donnait des amidons O-méthylés. Ces amidons O-méthylés possèdent un groupe méthyl à toutes ces positions qui antérieurement portaient des groupes xanthates.

Une analyse chromatographique des produits obtenus après une hydrolyse acide des amidons O-méthylés montrait que la D-glucose et ses éthers mono- et diméthyles étaient présents dans les proportions molaires 34:4:0,1 et 32:13:1 pour les faibles et forts degrés de substitutions respectivement. La résolution de la composante mono-O-méthyl du produit ayant un degré de substitution 0,33 montrait 67°/06-, 27°/02- et 6°/03- o-méthyl-D-glucose. Le produit avec le faible degré de substitution montrait 36°/06- et 44°/02-o-méthyl-D-glucose mais pas de 3-o-méthyl-D-glucose. La tritylation du xanthate S-benzyle ayant un degré de substitution de 0,33 confirmait le degré de substitution du xanthate primaire qui avait déjà été obtenu par analyse chromatographique.

**Literature Cited**

1. **DOANE, W. M., C. R. RUSSELL and C. E. RIST:** Stärke 17 (1965), 77.
7. **LENZ, R. W., and C. V. HOLMBERG:** Anal. chem. 28 (1956), 7.

**Address of the Authors:** W. M. DOANE, Ph.D., C. R. RUSSELL, Ph. D., and C. E. RIST, Cereal Products Laboratory, Northern Regional Research Laboratory* Peoria, Illinois (U.S.A.).

*) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(Received: April 20, 1965)