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Introduction of 3,6-Anhydro Rings into Amylose and Characterization of the Products[1]

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Received March 15, 1961

3,6-Anhydro rings are introduced into amylose by tosylation in pyridine and elimination of the resulting tosyloxy group with sodium methoxide in methanol, or by tritylation in pyridine, acetylation of the resulting 6-*O*-tritylamylose, detritylation with hydrochloric acid in methanol, tosylation of the resulting acetate, and final removal of both tosyloxy and acetyl groups with sodium methoxide in methanol. Analysis of the products shows that tosyloxy groups on primary carbon atoms are removed with quantitative formation of 3,6-anhydro rings. Complete methanolysis leads to isolation of crystalline methyl α-D-glucopyranoside, crystalline methyl β-D-glucopyranoside, and crystalline methyl 3,6-anhydro-β-D-glucofuranoside. From the results of partial methanolysis, which produces only a small proportion of disaccharide derivatives, a non-random mode of distribution of the anhydro rings along the chain of the macromolecule is indicated. Solubility behavior and rate of hydrolysis are also described.

Recent work shows that 3,6-anhydro rings occur in a variety of red-seaweed polysaccharides,[2] significant among which are agar,[3] carrageenan,[4] and the polysaccharide of *Gloiopeltis furcata*.[5] These polysaccharides have unusual properties which lend the carbohydrates to many practical uses.[6] It is of interest, therefore, to examine the changes in properties which occur when 3,6-anhydro rings are introduced into the amylose molecule. While this work was in progress, a brief report appeared[7] on 3,6-anhydroamylose, but no quantitative analyses or physical properties were reported. In the present synthesis, two routes for the introduction of 3,6-anhydro rings into amylose were developed.

One procedure employed tosylation of amylose with tosyl chloride (*p*-toluenesulfonyl chloride) in pyridine and subsequent elimination of the resulting tosyloxy group with sodium methoxide in methanol. It is likely that very little degradation of amylose ensued. Optimum reaction conditions were sought by examination of variations in temperature, reaction time, and quantities of tosyl chloride. An attempt was made to establish those conditions which led to maximum substitution of primary hydroxyl groups and minimum substitution of secondary hydroxyls. Three samples of carefully prepared amylose tosylate, designated A1, A2 and A3, are listed in Table I. It is evident that almost all substitution is on primary hydroxyl groups. Treatment of the tosyl esters with excess sodium methoxide in methanol afforded polysaccharides containing 3,6-anhydro rings and a slight proportion of unremoved tosyl groups.

TABLE I

TOSYL GROUPS AND 3,6-ANHYDRO RINGS IN 3,6-ANHYDROAMYLOSE, EXPRESSED AS DEGREES OF SUBSTITUTION

	Tosylamylose Tosyl Groups		3,6-Anhydroamylose		
	Primary	Secondary	Unremoved tosyl groups	Anhydro rings	
A1	0.60	0.06	0.03	0.64[a]	0.65[b]
A2	0.70	0.08	0.03	0.72	0.73
A3	0.86	0.13	0.07	0.88	0.89
B1	0.27	0.01	0.00	0.28	0.28
B2	0.57	0.04	0.01	0.57	0.55
B3	0.85	0.08	0.02	0.83	0.88

[a] Data in this column obtained by periodate oxidation. [b] Data in this column obtained by acetylation.

The second synthetic route involved tritylation of amylose with trityl chloride (chlorotriphenylmethane), followed by acetylation, detritylation, tosylation, and final saponification. This route is similar to that described by Bines and Whelan.[7] Under proper conditions of tritylation, a derivative is obtained with exactly one trityl group for each D-glucose unit. Since tritylation is more specific for primary hydroxyl groups than is tosylation, as was shown for cellulose by Honeyman,[8] the product can be assumed to be essentially 6-*O*-tritylamylose. After acetylation with acetic anhydride and pyridine the resulting 2,3-di-*O*-acetyl-6-*O*-tritylamylose was treated with a very dilute solution of hydrochloric acid in methanol to effect detritylation.[9] During this process, some acetyl groups were also removed. Analysis showed that the product was free of trityl groups but contained

(1) Journal Paper No. 1731 of the Purdue Agricultural Experiment Station. Presented at the 138th Meeting of the American Chemical Society, New York, N. Y., September 1960.

(2) C. Araki, *Proc. 4th International Congress of Biochemistry*, Vol. I, M. L. Wolfrom, ed., Pergamon Press, New York, 1959, p. 15.

(3) C. Araki, *Bull. Chem. Soc. Japan*, 29, 543 (1956).

(4) (a) A. N. O'Neill, *J. Am. Chem. Soc.*, 77, 2837, 6324 (1955); (b) C. Araki and S. Hirase, *Bull. Chem. Soc. Japan*, 29, 770 (1956).

(5) S. Hirase, C. Araki, and T. Ito, *Bull. Chem. Soc. Japan*, 29, 985 (1956); 31, 428 (1958).

(6) See *Industrial Gums*, R. L. Whistler, ed., Academic Press, Inc., New York, 1959.

(7) B. J. Bines and W. J. Whelan, *Chem. & Ind. (London)*, 997 (1960).

(8) J. Honeyman, *J. Chem. Soc.*, 168 (1947).

(9) W. M. Hearon, G. D. Hiatt, and C. R. Fordyce, *J. Am. Chem. Soc.*, 65, 2449 (1943).

1.7 acetyl groups for each D-glucose unit. In addition, some depolymerization of the amylose was inevitable and the average chain length decreased from 159 units to 145, as estimated by viscosity measurements.

The detritylated acetate was tosylated with tosyl chloride in pyridine and three samples of the mixed acetyl tosyl ester, designated B1, B2, and B3, are listed in Table I. In these derivatives tosylation of secondary hydroxyl groups occurred to a lesser extent than in the samples A1, A2, and A3. Saponification of the mixed esters with excess sodium methoxide introduced 3,6-anhydro rings, but the product still retained a very small proportion of tosyl groups (Table I).

The anhydroamylose preparations A1, A2, and A3 swell but do not dissolve in water, 4% sodium hydroxide solution, or common organic solvents. Preparation B1 is soluble in water, and preparations B2 and B3 are partly soluble in water and in alkali solution. Insolubility could result from extensive introduction of 3,6-anhydro rings, from uneven distribution of the anhydro rings, or from ether cross-links between chains.

Hydrolyzates of the anhydro preparations of the A series gave no paper-chromatographic evidence of di-D-glucose anhydrides which might arise if amylose molecules were cross-linked. Known di-D-glucose anhydrides were used for chromatographic comparisons. The water solubility of some of the anhydroamylose samples of the B series may result from degradation during preparation.

The existence of 3,6-anhydro rings in anhydroamylose was established by complete methanolysis, which led to isolation of crystalline methyl β-D-glucopyranoside, crystalline methyl β-D-glucofuranoside, crystalline methyl 3,6-anhydro-β-D-glucofuranoside, and a mixture of methyl 3,6-anhydro-α- and β-D-glucofuranosides; the mixture, on hydrolysis, afforded crystalline 3,6-anhydro-D-glucose. Furthermore, removal with alkali of tosyl groups residing on hydroxyls of C-2 or C-3 in amylose would form 2,3-anhydro rings with inversion of configuration, and the resulting anhydro rings would be reopened by the action of excess sodium methoxide, again with configurational changes to give 2- or 3-*O*-methyl-hexose residues.[10] However, none of the possible products could be detected chromatographically.

Amounts of the anhydro rings in the polysaccharide were estimated by determination of periodate consumption and by determination of the acetyl contents of the fully acetylated derivatives. The results are included in Table I. It is seen that the values obtained by these methods are in reasonable agreement and also agree with the degree of primary tosylation. It is evident that the tosyl groups on primary hydroxyl groups are removed, with quantitative formation of 3,6-anhydro rings.

As expected, introduction of 3,6-anhydro rings labilizes the glycosidic linkage[10] to acid hydrolysis. Hydrolysis of the anhydroamylose with 0.05*N* hydrochloric acid proceeds very rapidly during the first four hours, as compared with unmodified amylose; thereafter, the rate of hydrolysis becomes equivalent to the rate for amylose.

As the anhydroglucosidic linkages are very easily cleaved, partial methanolysis of anhydroamylose containing one anhydro ring for two D-glucose units would produce methyl 3,6-anhydro-4-*O*-(α-D-glucopyranosyl)-D-glucopyranoside or its corresponding dimethyl acetal as the principal product, if the anhydro rings were distributed uniformly along the chain. Similar types of disaccharide derivatives have been isolated in good yields from agar[11] and from the polysaccharides of *Chondrus*[4b] and *Gloiopeltis*.[5] With this in mind, the anhydroamylose samples prepared from the tosyl esters A1 and B2 were subjected to partial methanolysis and the products were separated on a charcoal Celite column.[12]

Paper chromatographic examination of the resulting fractions showed the unexpected presence of many components in each fraction. Fractions eluted from the column with 7.5% to 20% aqueous ethanol contained disaccharide derivatives in very small proportions. A fraction containing monosaccharide derivatives and fractions with higher oligosaccharide derivatives were obtained as major products. This might be expected if the anhydro rings were clustered at intervals of two or more D-glucose residues. Thus, tosylation of amylose may not be random, and a substituent on one D-glucose residue may influence the reactivity of its neighboring D-glucose residues so as to lead to clusters of esterified residues in various parts of the chain.

The charcoal column fraction eluted with 20% ethanol gave a substance melting at 163–165° and having an optical rotation of $[\alpha]_D^{25}$ +19.0°. Analyses and molecular weight determinations established the empirical formula $C_{12}H_{18}O_8(OCH_3)_2$. Each mole of the compound consumed 1.0 mole of periodate. Hydrolysis produced 3,6-anhydro-D-glucose and some 5-(hydroxymethyl)-2-furaldehyde, which arose from the 3,6-anhydro derivative during hydrolysis. These data suggest that the compound is 3,6-anhydro-2-*O*-(3,6-anhydro-D-glucopyranosyl)-D-glucose dimethyl acetal, but the presence of the 1→2 linkage indicated is unexplained

(10) See the review by S. Peat, *Advances in Carbohydrate Chem.*, 2, 37 (1946).

(11) C. Araki and S. Hirase, *Bull. Chem. Soc. Japan*, 27, 109 (1954); A. L. Clingman, J. R. Nunn, and A. M. Stephen, *J. Chem. Soc.*, 197 (1957).

(12) R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.*, 72, 677 (1950).

EXPERIMENTAL

Tosylation of amylose. In the reactions described, the products were precipitated and purified as follows. The viscous reaction solution was poured in a thin stream, with stirring, into a large excess of 80% aqueous methanol. The tosyl ester was precipitated, usually as a sticky gum, and separated from liquids either by decantation or centrifugation. After rinsing with 80% aqueous methanol, the gum was vigorously agitated in water in a Waring Blendor. This treatment converted the gum into a powder, which was collected on a Büchner funnel, washed free from chloride ion with water, and dried at 60° over calcium chloride in a vacuum oven.

Tosyl groups on primary and secondary hydroxyl groups were estimated by the iodination method[13] as follows. Total tosyl groups were estimated from sulfur analyses. Tosyloxy groups on primary hydroxyl groups were then replaced by iodine through reaction with sodium iodide in 2,5-hexanedione at 120° for 2 hr. The product was analyzed for iodine to determine those tosyl groups originally on primary positions, and then further analyzed for sulfur to determine tosyl groups remaining on secondary positions.

Sulfur analyses were done by the Carius procedure.[14] Iodine analyses were done by converting organic iodine into iodide with potassium hydroxide according to Mahoney and Purves,[15] with the modification that the digestion period was extended to 5 hr. and the resulting iodide was titrated iodometrically.[16]

Results of the use of 3.0*M* proportions of tosyl chloride per D-glucose unit at 35° for various lengths of time are shown in Table II. Ten grams (0.062 basic mole) of dry corn amylose was soaked in 200 ml. of dry pyridine at 35° for 30 min. with gentle stirring, and 35 g. (0.18 mole) of tosyl chloride was added in one portion. The amylose dissolved gradually during 30 min. A part of the thick solution was withdrawn at intervals, and the product was precipitated and purified. Most of the primary hydroxyl groups are substituted within the first hour, after which time substitution takes place gradually on secondary hydroxyl groups (Table II).

TABLE II

TOSYLATION OF AMYLOSE AT 35° WITH 3.0*M* PROPORTIONS OF TOSYL CHLORIDE PER D-GLUCOSE UNIT

Reaction Time, Hr.	Tosyl Groups per D-Glucose Unit	
	Primary	Secondary
0.5	0.86	0.14
1.0	0.94	0.20
2.0	0.96	0.29
5.0	0.91	0.39
10.0	0.90	0.52

A similar, but less distinct, result was obtained as shown in Table III, when the reaction was done in exactly the same manner as described above, except that 1.2*M* proportions of tosyl chloride per D-glucose unit were used. These combined results showed that the degree of substitution is dependent not only upon reaction time but also upon the proportion of tosyl chloride used.

TABLE III

TOSYLATION OF AMYLOSE AT 35° WITH 1.2*M* PROPORTIONS OF TOSYL CHLORIDE PER D-GLUCOSE UNIT

Reaction Time, Hr.	Tosyl Groups per D-Glucose Unit	
	Primary	Secondary
0.5	0.49	0.03
1.0	0.63	0.10
2.0	0.68	0.10
5.0	0.70	0.11
10.0	0.73	0.12

Results of reactions at 60° using 1.2*M* proportions of tosyl chloride per D-glucose unit are given in Table IV. Substitution took place very rapidly, followed by a detosylation. On the other hand, reaction at 3°, using 3.0*M* proportions of tosyl chloride per D-glucose unit, proceeded very slowly as shown in Table V. In this case, the reaction mixture was still heterogeneous after 48 hr.

TABLE IV

TOSYLATION OF AMYLOSE AT 60° WITH 1.2*M* PROPORTIONS OF TOSYL CHLORIDE PER D-GLUCOSE UNIT

Reaction Time, Hr.	Tosyl Groups per D-Glucose Unit	
	Primary	Secondary
0.5	0.76	0.07
1.0	0.66	0.10
2.0	0.66	0.09
5.0	0.57	0.10
10.0	0.51	0.12

TABLE V

TOSYLATION OF AMYLOSE AT 3° WITH 3.0*M* PROPORTIONS OF TOSYL CHLORIDE PER D-GLUCOSE UNIT

Reaction Time, Hr.	Tosyl Groups per D-Glucose Unit
5	0.06
10	0.11
24	0.23
48	0.32

It was concluded that optimum reaction conditions for primary tosylation were obtained when the mixture was held at 35° for 1 hr. Thus, 30 g. (0.185 mole of D-glucose unit) of amylose was tosylated under these conditions using 35 g. (0.18 mole), 42 g. (0.22 mole), and 70 g. (0.37 mole) of tosyl chloride to produce 42, 50, and 58 g. of product, respectively, designated A1, A2, and A3 in Table I. All the products were obtained as white powders; their degrees of substitution are given in Table I.

Elimination of tosyloxy groups of tosylamylose. In preliminary trial runs, four samples of the tosyl ester A3 (10.09% S), each weighing 0.5 g., were each placed in 10 ml. of 0.5*N* sodium methoxide in methanol and each mixture was shaken at 25°. Analyses showed that products isolated after 1, 2, 4, and 6 days of reaction contained 5.19, 3.55, 1.48, and 1.16% of sulfur, respectively; the last value is equivalent to 0.06 tosyl group per D-glucose unit. No further decrease in sulfur content was observed, even when the product isolated after 6 days of reaction was treated with fresh alkaline solution for an additional 2 days.

Thirty-five grams of the tosyl ester A1 and 530 ml. of absolute methanol containing 6.1 g. (three fold excess) of sodium

(13) F. B. Cramer and C. B. Purves, *J. Am. Chem. Soc.*, **61**, 3458 (1939); T. S. Gardner and C. B. Purves, *J. Am. Chem. Soc.*, **64**, 1539 (1942); C. J. Malm, L. J. Tanghe, and B. C. Laird, *J. Am. Chem. Soc.*, **70**, 2740 (1948).

(14) *Methods of Analysis—A.O.A.C.*, 8th Edition, W. Horwitz, ed., Association of Official Agricultural Chemists, Washington, D. C., 1955, p. 806.

(15) J. F. Mahoney and C. B. Purves, *J. Am. Chem. Soc.*, **64**, 9 (1942).

(16) I. M. Kolthoff and R. Belcher, *Volumetric Analysis*, Vol. III, Interscience, New York, 1957, p. 248.

were stirred at 35° for 6 days under dry nitrogen. The solid was then collected on a large sintered glass funnel and washed with 200 ml. of methanol and 250 ml. of 80% aqueous methanol. The product was vigorously agitated in methanol in a Waring Blendor to give a powder. Filtration, washing, and disintegration in a Waring Blendor were repeated until the washings became neutral to phenolphthalein. Anhydroamylose, dried at 40° in a vacuum, was obtained as a slightly colored powder weighing 15 g.

A similar treatment of the tosyl esters A2 and A3 afforded the corresponding anhydroamyloses as light brown powders. Yields were 28 g. from 52 g. of A2 and 21 g. from 38 g. of A3.

All three samples of the anhydroamylose obtained above swelled in but were insoluble in water, 4% sodium hydroxide, *N,N*-dimethylformamide, dimethyl sulfoxide, dioxane, ethylene glycol monomethyl ether, and ethylene glycol dimethyl ether. The residual tosyl groups are indicated in Table I.

Tritylation of amylose. Although tritylation of carbohydrates was once thought to be highly specific for primary hydroxyl groups, extensive studies of tritylation of cellulose showed that the amount of primary trityl depends upon the proportion of trityl chloride used and the reaction time.[8,9] Thus, initial experiments were done to find the reaction conditions under which one trityl group per D-glucose unit (as closely as possible) could be introduced into amylose.

Two grams of amylose and 30 ml. of absolute pyridine were heated in a closed vessel on a steam bath. When the temperature of the mixture reached 95°, a known weight of trityl chloride was added and the heating and stirring were maintained for a given length of time. In all cases, a homogeneous solution resulted after about 40 min. Methanol was then added, with stirring, to near the saturation point and, after being cooled to 25°, the solution was poured into 250 ml. of methanol with vigorous stirring. The precipitate was collected on a Büchner funnel and washed with methanol. Agitation in a Waring Blendor, followed by filtration and washing, was repeated until the washings were free from chloride ion. The product was dried over potassium hydroxide at 60° in a vacuum. The trityl content of the product was estimated by the method of Hearon, Hiatt, and Fordyce.[9]

Results obtained by varying the proportion of trityl chloride and lengths of reaction time are shown in Table VI.

TABLE VI

TRITYLATION OF AMYLOSE AT 100°

Moles Trityl Chloride per Mole D-Glucose Unit	Reaction Time, Hr.	Trityl Groups per D-Glucose Unit
1.5	1	0.75
	2	0.81
	5	0.86
	10	0.85
3.0	1	0.94
	2	1.01
	5	1.04
	10	1.06
5.0	1	0.95
	2	1.06
	5	1.08
	10	1.08

The product obtained by reaction with 3 moles of trityl chloride for 2 hr. contained very nearly one substituted group for reach D-glucose unit. Hence, 50 g. (0.31 basic mole) of amylose in 500 ml. of dry pyridine was treated with 260 g. (0.93 mole) of trityl chloride at 100° for 2.5 hr. Reaction and purification of the product were done as described above. The powdery product, 125 g. (100%), had a slight creamy color.

Anal. Calcd. for $[C_6H_9O_5(C_{19}H_{15})]_n$: trityl, 60.16. Found: 60.12.

Acetylation of 6-O-tritylamylose. To a solution of 121 g. (0.3 basic mole) of the above 6-*O*-tritylamylose in 250 ml. of dry pyridine was added 122 g. (1.2 moles) of acetic anhydride with shaking, and the mixture was heated on a steam bath for 3 hr. After being cooled to 25°, the viscous solution was poured portionwise into methanol stirred in a Waring Blendor. The precipitate was collected on a Büchner funnel, washed with methanol, and transferred to the Blendor with methanol for further disintegration. This process was repeated three times. The product finally collected on a Büchner funnel was washed thoroughly with methanol and dried at 40° in a vacuum. 2,3-Di-*O*-acetyl-6-*O*-tritylamylose was obtained as a white powder; yield 134 g. (92%).

Anal. Calcd. for $[C_6H_7O_5(CH_3CO)_2(C_{19}H_{15})]_n$: acetyl, 17.62; trityl, 49.82. Found: acetyl, 17.52; trityl, 50.73.

Detritylation of 2,3-di-O-acetyl-6-O-tritylamylose. The procedure described by Hearon and his co-workers[9] was followed with slight modification. A 50-g. portion of the di-*O*-acetyl-*O*-tritylamylose (40 mesh) was suspended in 750 ml. of methanol containing 3.5 ml. of concd. hydrochloric acid. The suspension was stirred at 25° for 7 hr., at which time the solid was filtered, and washed with 500 ml. of methanol. A 1-g. portion was further washed and dried. Analysis showed that it contained 24.77% trityl groups. The major portion was again treated for 7 hr. with the same quantity of the fresh reagent as that used above. As a sample at this point still showed 0.91% trityl content, the same treatment was repeated once more. The final product was obtained as a white powder amounting to 23 g. A sample dissolved in concentrated sulfuric acid showed a slight yellow color, but, on dilution with water, gave no precipitate of tritylcarbinol, indicating that detritylation was complete. Acetyl determination showed that some acetyl groups were also removed during the process, the resulting degree of substitution being 1.7.

Anal. Calcd. for $[C_6H_{8.3}O_5(CH_3CO)_{1.7}]_n$: acetyl, 31.27. Found: 31.17.

To determine the extent of degradation during the process, the acetate was treated with acetic anhydride and pyridine to give a fully acetylated derivative, the viscosity of which was then measured in pyridine solutions.[17] The intrinsic viscosity found was 27.6, equivalent to an average chain length of 145 using 0.7×10^{-4} as the K_m constant. A sample of tri-*O*-acetylamylose prepared directly from amylose had an intrinsic viscosity of 32.1 or an average chain length of 159.

Tosylation of di-O-acetylamylose. Three samples of tosylated di-*O*-acetylamylose, B1, B2, and B3, having different degrees of tosylation, were prepared by variation of the proportion of tosyl chloride. Fifteen grams of the approximate di-*O*-acetylamylose above was dissolved in 150 ml. of dry pyridine. To the solution kept at 35° was added dropwise, with stirring, a solution of tosyl chloride in 30 ml. of dry pyridine during 1 hr., the quantities of tosyl chloride used being 7.3 g. (0.6 mole per D-glucose unit) for B2 and 24.4 g. (2.0 moles per D-glucose unit) for B3. The solution was kept at 35° for 5 hr. and then poured into 1.5 l. of 80% aqueous methanol, whereupon the tosyl ester was precipitated as a gum. Each precipitate was triturated with 80% methanol and then vigorously agitated with water in a Waring Blendor until it became a solid powder, which was filtered, washed free from chloride ion with sufficient water, and dried at 60° in a vacuum. Yields from B1, B2, and B3 were 23, 20, and 18 g., respectively. The degree of substitution was estimated in the same manner as described earlier (Table I).

Saponification of acetyltosylamylose. The tosyl ester obtained above was dissolved in ten times its weight of absolute dioxane. The solution was placed in a Waring Blendor and 10 ml. of *N* sodium methoxide in methanol was added with

(17) J. W. Mullen, II, and E. Pacsu, *Ind. Eng. Chem.*, **34**, 1209 (1942).

vigorous agitation. The deacetylated product was precipitated immediately. A volume of *N* sodium methoxide in methanol equal to the dioxane used was now added. The mixture was stirred for 6 days at 25° under dry nitrogen. Isolation and purification of the product were done in the same way as described earlier. The yields of the anhydroamylose were 8.5, 8.5, and 8.0 g. from 18 g. of B1, 20 g. of B2, and 23 g. of B3, respectively. Unremoved tosyl groups are shown in Table I.

The anhydroamylose from B1 was soluble in water. Those from B2 and B3 were partly soluble both in water and in 4% sodium hydroxide solution. All three samples swelled in but were insoluble in *N,N*-dimethylformamide, dimethyl sulfoxide, dioxane, ethylene glycol monomethyl ether, and ethylene glycol dimethyl ether.

Periodate oxidation of anhydroamyloses. An approximately 0.1-g. sample was mixed with 2.0 ml. of 0.491*M* solution of sodium metaperiodate, and the mixture was kept in the dark at 25° with occasional shaking. After an appropriate time had elapsed, the residual oxidant was iodometrically titrated in the usual manner. The results are shown in Table VII. From the figures obtained after 20 hr., the amounts of anhydro ring per D-glucose unit were calculated; they are listed in Table I.

TABLE VII

PERIODATE OXIDATION OF 3,6-ANHYDROAMYLOSES

Anhydroamylose Sample	Periodate Consumed, Millimoles per G., Hr.			
	2	5	10	20
A1	2.11	2.31	2.40	2.42
A2	1.62	1.80	1.87	1.88
A3	0.58	0.78	0.82	0.82
B1	4.46	4.60	4.61	4.61
B2	2.66	2.82	2.84	2.84
B3	1.01	1.10	1.13	1.15

Acetylation of anhydroamyloses. A 1-g. portion of the anhydroamyloses from B1, B2, and B3 was treated with 5.6 g. of dry pyridine and 4.8 g. of acetic anhydride in the usual manner. Double amounts of the reagents were needed for the acetylation of samples from A1, A2, and A3, as, otherwise, the reaction mixture became thick, due to the insolubility of the products. From the acetyl[18] contents of the products, the amounts of anhydro ring per D-glucose unit were calculated (Table I).

Hydrolysis of anhydroamyloses. A sample of about 0.6 g. was heated with 40 ml. of 0.05*N* hydrochloric acid on a steam bath. A part of the solution was withdrawn at intervals, neutralized with 0.1*N* sodium hydroxide solution, and then analyzed for reducing sugars by the Willstätter and Schudel method. Hydrolysis was found to be complete in about 4 hr.

Complete methanolysis of anhydroamylose. A 2-g. sample of the anhydroamylose (0.7 anhydro ring per D-glucose unit) from A2 was heated under reflux with 50 ml. of *N* hydrogen chloride in methanol for 30 hr. The resulting solution was neutralized with silver carbonate, filtered, and evaporated under reduced pressure to a sirup, which amounted to 2.3 g.; $[\alpha]_D^{25}$ +54.2° (*c*, 1.56 in water). When paper chromatographed with 1-butanol-ethanol-water (40:11:19 v./v.) as irrigant and sprayed with an *o*-aminophenol reagent,[19] it showed components with $R_{glucose}$ values of 2.49 and 2.78, corresponding to methyl 3,6-anhydro-β-D-glucofuranoside and possibly its α anomer, respectively.

The sirup obtained above was chromatographed on a charcoal-Celite column[12] (5.0 × 23 cm.) and separated into three fractions. Fraction I, eluted from the column with 4 l. of water and then 2 l. of 1.5% ethanol in water, was shown to be a mixture of methyl α- and β-D-glucopyranosides; yield 0.51 g. (22% of the methanolyzate); $[\alpha]_D^{25}$ +120.5° (*c*, 1.0 in water). It gave no spot on a paper chromatogram when sprayed with an *o*-aminophenol reagent. Crystallization from 95% ethanol afforded methyl α-D-glucopyranoside in two crops in a total yield of 0.32 g.; m.p. 163–165°. On recrystallization from 95% ethanol: m.p. and mixed m.p. 168–169°, $[\alpha]_D^{25}$ +159.1° (*c*, 1.0 in water). Evaporation of the mother liquor afforded the β anomer in a yield of 0.09 g.; m.p. 103–105°. Recrystallization from 99% ethanol gave the pure compound; m.p. 104–106°, $[\alpha]_D^{25}$ −34.0° (*c*, 1.0 in water). The filtrate, separated from the crystalline compounds, was evaporated to a sirup (0.10 g.), which was then hydrolyzed with *N* sulfuric acid solution at 100° for 4 hr. Paper chromatographic examination showed that the hydrolysis product was mainly D-glucose contaminated with a small proportion of 3,6-anhydro-D-glucose.

Fraction II, eluted from the column with an additional 2 l. of 1.5% aqueous ethanol and 6 l. of 5% aqueous ethanol, was proved to be a mixture of methyl 3,6-anhydro- β-D-glucofuranoside and possibly its α anomer; yield 1.25 g. (54% of the methanolyzate); $[\alpha]_D^{25}$ +33.2° (*c*, 1.0 in water). Paper chromatographic examination, using the same solvent mixture and spray as mentioned above, showed two spots, corresponding to methyl 3,6-anhydro-α- and β-D-glucofuranoside. It was taken up in ethyl acetate, a small amount of insoluble material was removed by filtration, and the filtrate was evaporated under reduced pressure to a sirup. Methyl 3,6-anhydro-β-D-glucofuranoside was crystallized by dissolving the sirup in a small volume of ethyl acetate, adding absolute ether dropwise until a slight turbidity formed, and placing the resulting solution in a refrigerator; yield 0.58 g.; m.p. 96–97°. Recrystallization from a mixture of ethyl acetate and ether afforded the pure compound; m.p. 97–98°, $[\alpha]_D^{25}$ −53.4° (*c*, 1.0 in water). Reported[20] for methyl 3,6-anhydro-β-D-glucofuranoside: m.p. 98°, $[\alpha]_D^{20}$ −54° (water). The filtrate from the crude crystals was evaporated under reduced pressure to a sirup (0.65 g.), which was then subjected to hydrolysis with 0.1*N* sulfuric acid solution at 100° for 2 hr. The product was crystallized from ethanol–ethyl acetate–petroleum ether (b.p. 60–68°) (1:1:1v./v.), giving 3,6-anhydro-D-glucose; yield 0.50 g.; m.p. 118–119°, $[\alpha]_D^{25}$ +55.1° (*c*, 1.0 in water). Accepted values: m.p. 118°, and $[\alpha]_D$ +53.8° (water).[20,21]

Fraction III was eluted from the column with 4 l. of 15% aqueous ethanol followed with 4 l. of 25% aqueous ethanol; yield 0.28 g. (12% of the methanolyzate); $[\alpha]_D^{25}$ +57.8° (*c*, 1.0 in water). It showed a series of spots on a paper chromatogram, the solvent and spray being the same as before. From this observation, coupled with the fact that a higher concentration of ethanol in water was used for its elution from the charcoal column, fraction III seemed to be a mixture of oligosaccharide derivatives. It was, therefore, subjected to complete hydrolysis with 2*N* sulfuric acid solution at 100° for 24 hr. On applying paper chromatography, the product showed components corresponding to D-glucose, 3,6-anhydro-D-glucose, and 5-(hydroxymethyl)-2-furaldehyde, the last compound probably arising from the second during hydrolysis. No product which would indicate a cross-linkage in the polysaccharide could be detected.

The anhydroamylose sample prepared from B2 gave a similar result on methanolysis. The yields of the fractions corresponding to fractions I, II, and III described above were 10, 76, and 8% of the methanolysis products, respectively. Methyl α-D-glucopyranoside, methyl β-D-glucopyranoside,

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and methyl 3,6-anhydro-β-D-glucofuranoside were obtained again as crystals in the same way as described previously.

Partial methanolysis of anhydroamylose. A 4-g. sample of anhydroamylose prepared from A1 was heated under reflux with 50 ml. of 0.1*N* hydrogen chloride in methanol for 3 hr. The acid was removed by neutralization with silver carbonate and filtration, and the filtrate was concentrated to a sirup which weighed 4.2 g. It was chromatographed on a charcoal Celite column (5 × 23 cm.), using successively increasing concentrations of ethanol in water as eluent. Five liters of each eluent was used. The results are shown in Table VIII.

TABLE VIII

CHROMATOGRAPHIC SEPARATION OF PARTIAL-METHANOLYSIS PRODUCTS OF ANHYDROAMYLOSE

Fraction	Eluent[a]	Yield, %	$R_{glucose}$[b]
I	W, and 5% E	43	2.49, 2.78
II	7.5% E	1	1.17, 2.49, 2.78
III	15% E	6	1.78, 2.37
IV	20% E	10	1.08, 1.54, 2.37
V	30% E	13	0.94, 1.19, 1.54
VI	50% E	18	At least 7 spots

[a] W = water; E = ethanol. [b] Paper chromatogram was developed with 1-butanol-ethanol-water (40:11:19 v./v.) and sprayed with *o*-aminophenol reagent.[19]

On the basis of the result of complete methanolysis described above, fraction I is probably a mixture of monosaccharide derivatives. Since fractions V and VI were eluted with 30% and 50% ethanol, these are probably mixtures of higher oligosaccharide derivatives. Disaccharide derivatives would be expected in fractions II, III, or IV; these fractions were obtained in lower yields. Among the several spots observed on paper chromatograms of fractions III and IV, a spot showing an $R_{glucose}$ value of 2.37 seemed to be the most intense. This component was isolated and characterized as described below.

Fractions III and IV were combined, and then separated on several chromatographic papers, the solvent being 1-butanol-ethanol-water (40:11:19 v./v.). The component having an $R_{glucose}$ value of 2.37 was recovered as a sirup which weighed 0.30 g. (7% of the methanolysis products). Crystallization was effected by dissolving it in a small volume of acetone, adding ether until a slight turbidity formed, and placing the resulting solution in a refrigerator; yield 0.21 g.; m.p. 158–160°. The product was twice recrystallized from a mixture of methanol and ether; m.p. 163–165°, $[\alpha]_D^{25}$ +19.0° (*c*, 1.0 in water).

Anal. Calcd. for $C_{12}H_{16}O_6$ $(OCH_3)_2$: C, 47.72; H, 6.87; OCH_3, 17.62; mol. wt., 352.3. Found: C, 47.92; H, 6.77; OCH_3, 17.46; mol. wt. (Rast), 350.

A 10.30-mg. portion of the crystals obtained above was dissolved in 10 ml. of 0.11*M* sodium metaperiodate solution and the mixture was kept in the dark at room temperature. The residual oxidant was titrated at intervals in the usual manner. The compound consumed 0.99, 1.01, and 1.03 moles of periodate after 7, 15, and 48 hr., respectively.

A 50-mg. portion of the crystals was hydrolyzed with 2 ml. of 0.05*N* sulfuric acid at 100° for 5 hr. The solution was neutralized with barium carbonate, filtered, and evaporated to a sirup (41 mg.). Paper chromatographic examination showed the presence of 3,6-anhydro-D-glucose and 5-(hydroxymethyl)-2-furaldehyde. Crystallization from ethanol–ethyl acetate–petroleum ether (1:1:1 v./v.) afforded 3,6-anhydro-D-glucose; yield 30 mg. (64%); m.p. and mixed m.p. 118°, $[\alpha]_D^{25}$ +54.0° (*c*, 0.5 in water).

A similar result was obtained when anhydroamylose prepared from B2 was subjected to partial methanolysis. The yields of chromatographic fractions were 41, 2, 9, 10, 15, and 17% of the methanolyzate, corresponding to fractions I, II, III, IV, V, and VI in Table VIII, respectively.

Acknowledgment. The authors thank the National Science Foundation and the Corn Industries Research Foundation for grants in partial support of this work.

LAFAYETTE, IND.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

2,3,5-Tri-*O*-benzyl-D-ribosyl and -L-arabinosyl Bromides

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Received March 22, 1961

In the D-ribofuranose and L-arabinofuranose series fully benzylated methyl furanosides have been synthesized. Mild acid hydrolysis converts these into 2,3,5-tri-*O*-benzylpentofuranoses, the L-arabinose isomer being obtained in crystalline form. *p*-Nitrobenzoylation of the isomer in each series gave 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoylpentoses, one anomer being obtained in crystalline form in the D-ribofuranose series and both in the L-arabinofuranose series. With a slight excess of hydrogen bromide in methylene chloride these esters were converted to the 2,3,5-tri-*O*-benzylpentosyl bromides. In both series, these highly reactive halides were obtained as sirups. The nature of the D-ribofuranosyl halide was demonstrated through reduction to the known 1,4-anhydro-D-ribitol as well as conversion to the anomeric methyl D-ribofuranosides. The L-arabinofuranosyl bromide was reduced to amorphous 1,4-anhydro-L-arabitol which was characterized as its crystalline tri-*p*-nitrobenzoate. The identity of this ester was confirmed through an independent synthesis from ethyl 5-*O*-benzoyl-1-thio-β-L-arabinoside. Improvements in the preparation of methyl β-D-ribofuranoside are described.

Although the acylated glycosyl halides are vital intermediates in a wide variety of syntheses, their usefulness has certain limitations. First, displacement of the halogen atom often takes place with participation of one of the acyl groups, resulting in the dominance of one anomer among the products. Second, the acylated glycosyl halides are generally unsuitable for the synthesis of those glycosyl derivatives in which alkali-labile substituents are attached to the reducing carbon, the removal of the masking acyl groups tending to eliminate the desired substituent. For these reasons