Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

METHANOLYSIS OF AMYLOSE TRIESTERS AND RELATED COMPOUNDS

GUSTAV ENTLICHER* AND J. N. BEMILLER
Department of Chemistry, Southern Illinois University, Carbondale, Illinois 62901 (U. S. A.)
(Received July 2nd, 1970)

ABSTRACT

Methanolysis, in acidic chloroform–methanol solutions, of a variety of esters of methyl α- and β-D-glucopyranosides, maltose, methyl β-maltoside, cellobiose, methyl β-cellobioside, and amylose revealed that (1) esterification stabilizes the glycosidic bond to methanolysis, so that no methanolysis occurs until extensive, or perhaps complete, deacylation of an acylated D-glucopyranosyl group has occurred; (2) methanolysis gives predominant, or perhaps complete, inversion; (3) methanolysis of the glycosidic bonds of amylose is more facile than hydrolysis; and (4) deacylation of acylated D-glucopyranosyl groups results in a significant change in optical rotation.

INTRODUCTION

During searches for a means of preparing specifically substituted methyl malto-oligosaccharides[1], various amylose triesters were subjected to methanolysis. It was found in these cursory investigations that amylose triacetate is rapidly methanolyzed to methyl α,β-D-glucopyranoside. This finding eventually led to the preparation of alkyl α-D-glucopyranosides *via* the alcoholysis of amylose triacetate and related compounds[2]. Higher esters of amylose were found to undergo methanolysis more slowly, but this reaction is still many times faster than the hydrolysis of amylose at the same temperature and acid concentration. Amylose tribenzoate was found to be insoluble in all solvents tested, and to be completely stable to methanolysis during several weeks of heating under the same conditions. The present paper describes detailed investigations of the methanolysis of amylose triesters.

RESULTS AND DISCUSSION

The change of optical rotation observed during the methanolysis of amylose triacetate, amylose tripropionate, and amylose tributyrate with 0.5M methanesulfonic acid in methanol–chloroform does not follow first-order kinetics (see Fig. 1). For the latter two esters, a rapid, initial decrease in positive rotation is followed by a plateau

*Present address: Department of Biochemistry, Charles University, Albertov 2030, Praha 2, Czechoslovakia.

in which there is little or no change in rotation; this, in turn, is followed by another rapid decrease in positive rotation. Methanolysis of amylose triacetate does not show the second change in optical rotation, because deacetylation, which is 60–75% complete in 2 h, occurs to such an extent that, at 4.5 h, precipitation of iodine-staining amylose terminates polarimetric measurements. The rates of change of rotation for the first portion of the curves are in the same sequence as that predicted for the rates of acid-catalyzed deacylations[3], namely, acetate > propionate > butyrate. The initial appearance of free methyl D-glucosides in the methanolysis of amylose triacetate was observed at 4 h, and this free D-glucoside consisted of ~97% of the β-D-pyranoside. Almost identical overall curves were obtained for all three esters when 0.5M hydrogen chloride was used instead of 0.5M methanesulfonic acid as the catalyst.

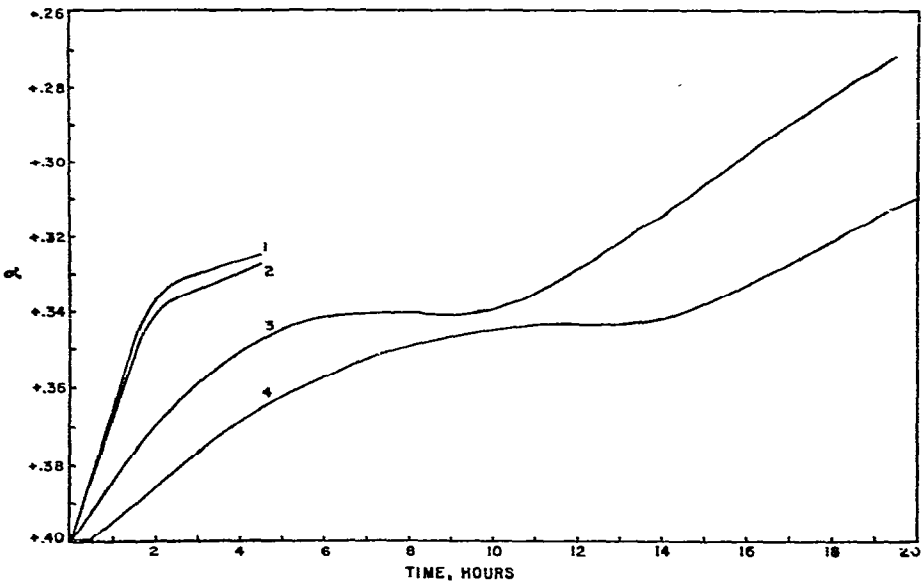


Fig. 1. Changes in observed optical rotation during reaction with acidic methanol–chloroform of (1) amylose triacetate, (2) amylose triacetate methanolyzed for 2.25 h and reacetylated, (3) amylose tripropionate, and (4) amylose tributyrate. Conditions are described in the text.

Several possibilities for the two distinct changes in optical rotation were investigated. (*1*) One hypothesis was that two separate methanolytic reactions were occurring, the first at the esterified, reducing end and the second at the glycosidic linkages. This possibility was investigated in two ways. In the first, amylose triacetate was methanolyzed for 2.25 h, at which time the first change was complete, and then carefully reacetylated under mild conditions and subjected to the same conditions of methanolysis (see Fig. 1). This second methanolysis gave a change in rotation that was practically identical with the first, indicating that methanolysis of an anomeric acetate to form a methyl D-glucoside at the reducing end is not responsible for the first change in rotation.

In the second investigation, compounds known to be esterified at the reducing end were methanolyzed (see Fig. 2). β-D-Glucopyranose pentaacetate gave a rapid, initial increase in positive rotation. The small proportion of completely deacetylated D-glucose that was observed by g.l.c. analysis at this time consisted entirely of α-D-

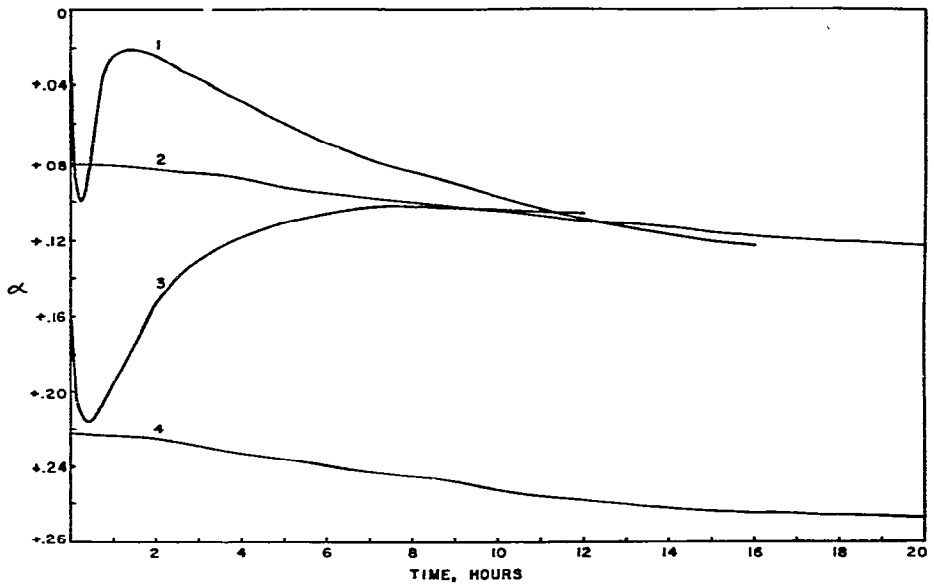


Fig. 2. Changes in observed optical rotation during reaction with acidic methanol–chloroform of (1) β-D-glucopyranose pentaacetate, (2) β-D-glucopyranose pentabenzoate, (3) β-maltose octaacetate, and (4) maltose octabenzoate. Conditions are described in the text.

glucopyranose, indicating a rapid anomerization; such acid-catalyzed anomerizations are well substantiated. The initial change was followed by a rapid and marked decrease in positive rotation. All of the (completely deacetylated) methyl D-glucoside present at this time was found to be methyl β-D-glucopyranoside. The decrease in positive rotation was followed by a slower increase in positive rotation, as the product was converted into an anomeric mixture of D-glucosides. β-Maltose octaacetate (curve 3 in Fig. 2), which has both an acetic ester group at the reducing end and an α-D-(1→4)-glucosidic linkage, gives a change in rotation similar to that for β-D-glucopyranose pentaacetate, with the exception of an increase in positive rotation after 90 min. This study, too, suggested that methanolysis of an acetate group on C-1 does not contribute significantly to the change in optical rotation observed for methanolysis of amylose triacetate.

Methanolysis of other model compounds was investigated. Methyl α-D-glucopyranoside tetraacetate (curve 2 in Fig. 3) gives a change in rotation that is reminiscent of the first portion of the curve for the methanolysis of amylose triacetate. Methyl β-maltoside heptaacetate (curve 5 in Fig. 3) gives a curve having a slight break at ~2 h, reminiscent of the overall curve for amylose triacetate.

β-D-Glucopyranose pentabenzoate (see Fig. 2) shows only a slow increase in positive rotation under the methanolysis conditions described; also, maltose octabenzoate (Fig. 2) does not show the rapid, initial anomerization characteristic of β-maltose octaacetate. For methyl α-D-glucopyranoside (see Fig. 3) under the same conditions, there is almost no change in optical rotation.

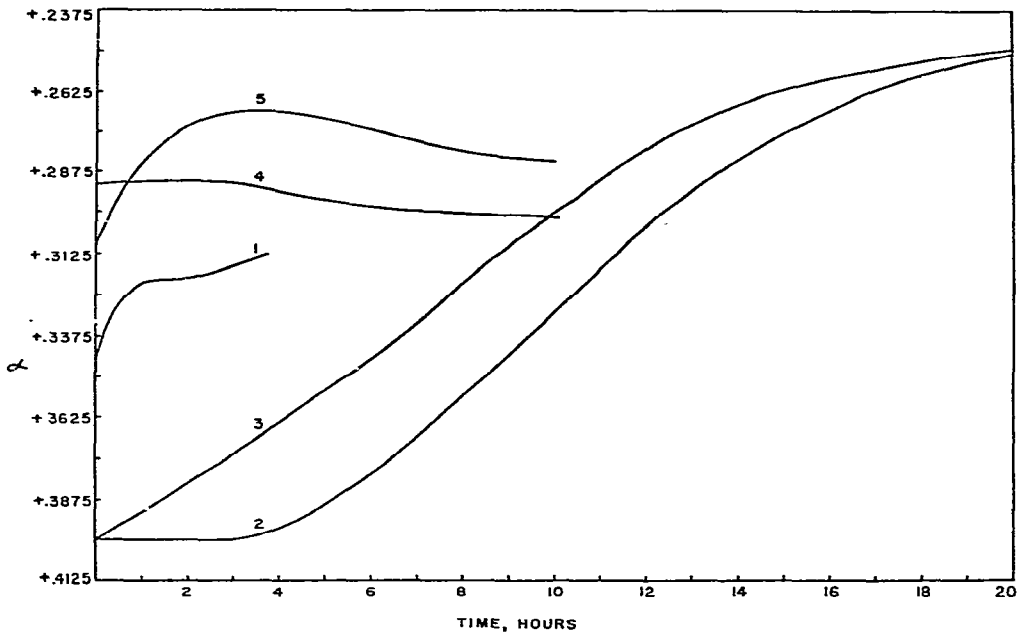


Fig. 3. Changes in observed optical rotation during reaction with acidic methanol–chloroform of (1) methyl α-D-glucopyranoside, (2) methyl α-D-glucopyranoside tetraacetate, (3) methyl α-D-glucopyranoside tetrabenzoate, (4) methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzoyl-α-D-glucopyranoside, and (5) methyl β-maltoside neptaacetate. Conditions are described in the text.

(*2*) A second explanation for the two distinct changes in optical rotation was that the first is associated with deacylation, and the second, with methanolysis of glycosidic linkages, and that methanolysis occurs only after deacylation. This possibility is supported by the fact that the rate of the first change in optical rotation in the methanolysis of amylose triacetate, tripropionate, and tributyrate (see Fig. 1) is directly related to the stability of the esters to acid-catalyzed hydrolysis[3]. A study of specific sites involved in this change was therefore made.

First investigated were derivatives in which the 2- and 3-hydroxyl groups were acetylated, and the 6-hydroxyl group was esterified with a more stable group. 2,3-Di-*O*-acetyl-6-*O*-*p*-tolylsulfonylamylose (see Fig. 4), under the methanolysis conditions, gave a smooth, slow change in optical rotation. After a lag period of 3 h, 2,3-di-*O*-acetyl-6-*O*-benzoylamylose (see Fig. 4) gave the same kind of curve. Monitoring of the debenzoylation of this derivative by the appearance of methyl benzoate revealed that no methyl benzoate was formed until the elapse of 3 h, and then its concentration increased steadily throughout the reaction, paralleling the decrease in positive rotation.

With both derivatives, sufficient ester remained to keep the reactant soluble in the chloroform–methanol solvent*.

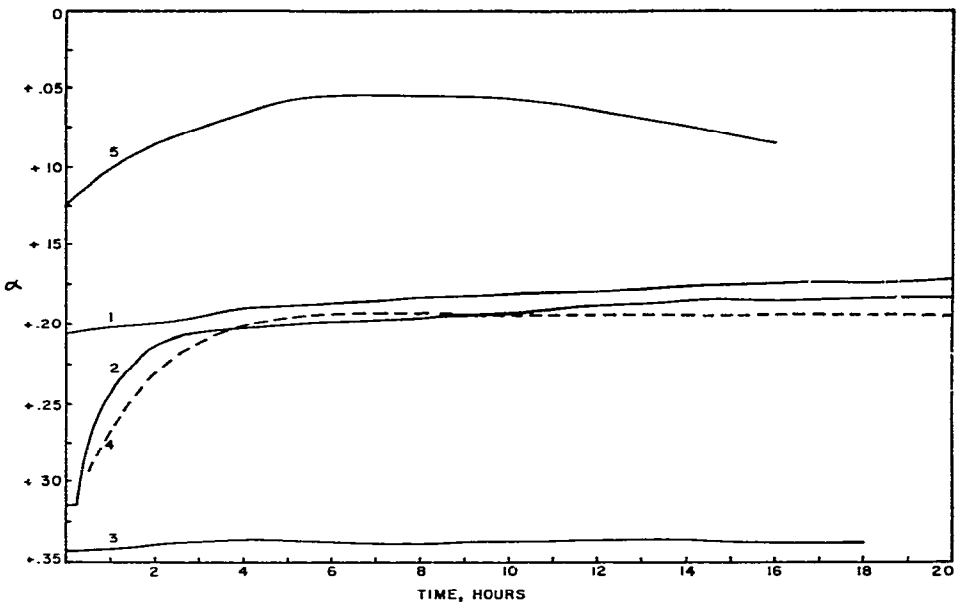


Fig. 4. Changes in observed optical rotation during reaction with acidic methanol–chloroform o (1) 2,3-di-*O*-acetyl-amylose, (2) 2,3-di-*O*-acetyl-6-*O*-benzoylamylose, (3) 2,3-di-*O*-acetyl-6-*O*-*p*-tolylsulfonylamylose, (4) 2,3-di-*O*-benzoylamylose, and (5) 6-*O*-acetyl-2,3-di-*O*-benzoylamylose. Conditions are described in the text.

A parallel experiment in which the deacetylation of amylose triacetate was examined by monitoring the formation of methyl acetate showed that no additional methyl acetate was formed after 8 h, indicating complete deacetylation at this time. Hence, with the 6-benzoate and 6-*p*-toluenesulfonate, the two portions of the curve observed with amylose triacetate, tripropionate, and tributyrate are coalesced into one, and the change in rotation is associated with the removal of the ester group from O-6. A model compound, namely, methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzoyl-α-D-glucopyranoside (see Fig. 3), showed an identical reaction, but the change in rotation was much more rapid.

To investigate further the hypothesis that removal of an ester group from O-6 results in a change in rotation, methanolysis of 6-*O*-acetyl-2,3-di-*O*-benzoylamylose (see Fig. 4) was undertaken. This reaction showed an initial decrease in positive rotation during the period of time in which removal of the acetyl group would be expected, and then a slow increase. That removal of acetyl groups from O-2 or O-3, or both, also contributes to the initial decrease in positive rotation during the methanolysis of amylose triacetate is shown by the results of methanolysis of amylose 2,3-diacetate (see Fig. 4). The corresponding amylose 2,3-dibenzoate (Fig. 4) shows a

* See Note added in proof on p. 373.

very slow increase in positive rotation. It should be noted that the positive, initial rotation of these compounds is in the order: amylose triacetate > amylose 2,3-diacetate > amylose 6-acetate 2,3-dibenzoate > amylose 2,3-dibenzoate.

An investigation of the incorporation of ^{14}C from methanol-^{14}C during the methanolysis of amylose tripropionate showed that incorporation begins at 7 h and increases steadily thereafter, again indicating that methanolysis of glycosidic bonds does not occur until extensive deacylation has taken place. This result excludes the possibility that acyl-group participation is responsible for the rapid-reaction rates and suggests the operation of a mechanism other than that involved in hydrolysis.

It was then decided to ascertain whether this change in rotation on deacylation is peculiar to α-D-glucopyranose residues. Accordingly, a series of compounds containing β-D-glucopyranose residues was subjected to methanolysis (see Fig. 5).

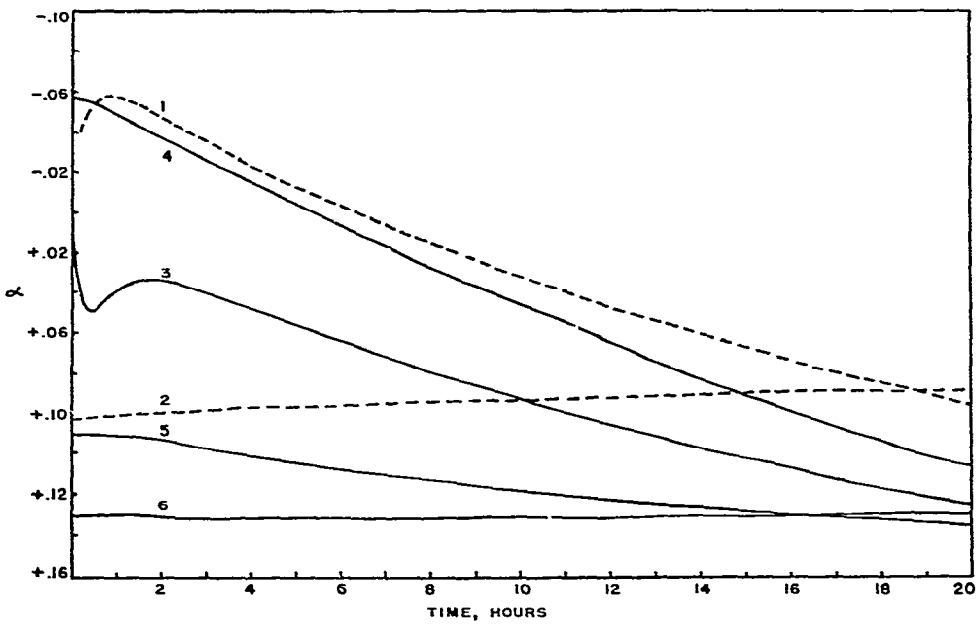


Fig. 5. Changes in observed optical rotation during reaction with acidic methanol–chloroform of (1) methyl β-D-glucopyranoside tetraacetate, (2) methyl β-D-glucopyranoside tetrabenzoate, (3) cellobiose octaacetate, (4) methyl β-cellobioside heptaacetate, (5) cellobiose octabenzoate, and (6) methyl β-cellobioside heptabenzoate. Conditions are described in the text.

Methyl β-D-glucopyranoside tetrabenzoate showed a very slow, but steady, decrease in positive rotation. Cellobiose octaacetate showed initial anomerization, next, the formation of methyl β-D-glucopyranoside, and then a slow, steady increase in positive rotation paralleling that occurring in the methanolysis of methyl β-cellobioside heptaacetate. Cellobiose octabenzoate showed a slow increase in positive rotation, due, perhaps, to anomerization, as methyl β-cellobioside heptabenzoate showed no change in optical rotation during the 20-h reaction period. A commercial sample of cellulose triacetate afforded a precipitate after less than 1 h of reaction.

These data suggested that deacylation of compounds containing α-D-glucopyranose residues results in a change in optical rotation, but, for compounds containing β-D-glucopyranose residues, the effect of deacylation on optical rotation is less clear. Hence, molecular rotations were calculated from data in the literature (see Table I). Although the rotations reported in this table were measured in solvents

TABLE I

SPECIFIC AND MOLECULAR ROTATIONS OF SOME METHYL D-GLUCOSIDES AND THEIR ESTERS[a]

Compound	*Solvent*	$[\alpha]_D$, *degrees*	[M], *degrees*	*References*
Methyl α-D-glucopyranoside	H_2O	+158	+30,700	4
2,3,6-triacetate		+110	+35,200	5
2,3-diacetate		+138	+38,300	5
2,3-diacetate 6-benzoate		+103	+39,200	5
tetraacetate	$CHCl_3$	+130	+47,200	6
tetrabenzoate	$CHCl_3$	+84	+51,200	7
2,3-dibenzoate	$CHCl_3$	+166	+66,600	8
2,3,6-tribenzoate	$CHCl_3$	+141	+71,400	8
Methyl β-D-glucopyranoside	H_2O	−34	−6,600	4
2,3,6-triacetate	$CHCl_3$	−59	−18,900	9
2,3-diacetate	$CHCl_3$	−52	−14,400	10
2,6-dibenzoate	$CHCl_3$	−29	−11,700	11
tetraacetate	$CHCl_3$	−18	−6,600	6
tetrabenzoate	$CHCl_3$	+31	+18,900	7
2,3-dibenzaoate	$CHCl_3$	+93	+37,300	9
2,3,6-tribenzoate	$CHCl_3$	+82	+41,500	9

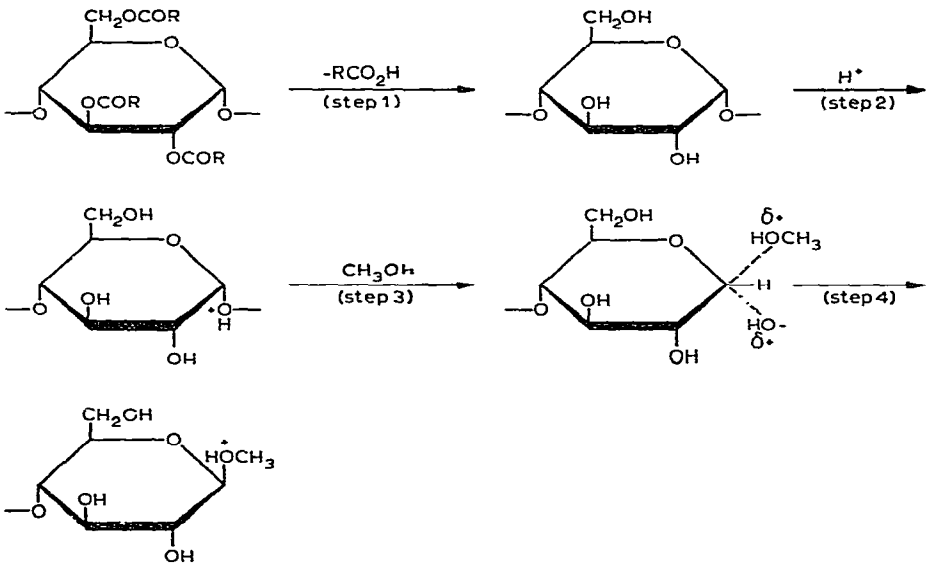
[a]From G. N. Bollenback, "Methyl Glucoside", Academic Press Inc., New York (1958).

other than that used in the present study, the data suggest that there may be a change in rotation upon deacylation of esters of methyl D-glucopyranoside and that, whatever the effect of acetylation on the optical rotation may be, it is much more pronounced for benzoylation. It will also be noted that, both in this work and in that collected in Table I, the compounds have the same order of positive, initial rotation, giving confidence to our use of the literature data as an indication of the effect of acylation on optical rotation.

No direct information is as yet available on the conformation of the D-glucopyranose residues present in amylose triacetate; n.m.r. evidence indicates that, in solution, methyl α-D-glucopyranoside tetraacetate adopts the *C1*(D) conformation[12]. For methyl 2-deoxy-α-L- and 3-deoxy-β-L-*erythro*-pentopyranoside, it has been determined that the changes in optical rotation that occur on dissolution in a variety of solvents result almost exclusively from changes in the conformational equilibria[13]. The conformational equilibria of the anomeric D-xylopyranose tetraacetates, compounds having the same configurations at C-2, C-3, and C-4 as the anomers of D-glucopyranose pentaacetate, have been determined[14]. It was found that, in solution, α-D-xylopyranose tetraacetate is present almost exclusively in the *C1*(D)

conformation at 28°, and that, for β-D-xylopyranose tetraacetate, *C1:1C* = 4.0:1.0. From this work, it may be speculated that deacylation of D-glucopyranoside esters results in a change in the equilibrium between conformations.

The conclusions may therefore be reached that (1) no methanolysis of amylose triesters occurs until extensive deacylation has taken place; (2) deacylation of amylose triacetate, tripropionate, and tributyrate is rather rapid, and precedes methanolysis of the glycosidic linkages; (3) removal of the benzoyl group from amylose 2,3-diacetate 6-benzoate is slow and concurrent with methanolysis; (4) methanolysis gives predominant inversion at C-1, as it does with tri-*O*-methylamylose[15]; and (5) methanolysis of the glycosidic bonds of amylose is much more facile than their hydrolysis. The following mechanism is proposed, in which rotational changes would occur at steps 1, 3, and 4.



In acidic, aqueous ethanol, sucrose octaacetate undergoes hydrolysis much more slowly than sucrose, and the degree of stability of ethoxycarbonyl derivatives of sucrose is directly related to the degree of substitution (D.S.)[16]. A derivative having D.S. 3.1 was very stable, one with D.S. 4.9 was completely stable under the same conditions, and octa(ethoxycarbonyl)sucrose was even stable in concentrated sulfuric acid.

As glycosidic bonds can be stabilized to methanolysis by benzoylation of the sugar units, it seemed possible that perbenzoylation of a glycoprotein followed by methanolysis of the peptide bonds might, perhaps, be used for isolating, as the perbenzoyl derivative, the carbohydrate component and the amino acid to which it is bound. This hypothesis was investigated by hydrolyzing both ovalbumin and glucoamylase I with pronase, isolating the glycopeptide fragments by gel filtration, benzoylating the glycopeptides, and subjecting the derivatives to the methanolysis conditions.

After 44 h, traces of free sugars, but no amino acids, were found, indicating that both the peptide bonds and the glycosidic bonds were quite stable to methanolysis.

EXPERIMENTAL

Methanolysis. — All of the carbohydrate esters used in this work were individually dissolved in chloroform. To this stock solution of each was added a solution of methanesulfonic acid in methanol to give a 3:1 (v/v) chloroform–methanol solution that was 34.7mM in carbohydrate (based on an "anhydro-D-glucopyranosyl" unit) and 0.5M in methanesulfonic acid. This solution was placed in an all-glass, jacketed, flow-through cell (preheated to 50°) in a Bendix ETL-NPL Automatic Polarimeter equipped with a 546-nm (mercury green line) interference filter.

Free methyl α-D-glucopyranoside was dissolved in hot methanol; chloroform was added, and the solution was cooled. A solution of methanesulfonic acid in methanol was then added, to give the same conditions (molar concentration of sugar, final concentration of acid, and chloroform to methanol ratio) as were used for the methanolysis of the sugar esters.

Esters. — Amylose triacetate, tripropionate, tributyrate, and tribenzoate were prepared by the usual procedure[17,18] and vacuum dried. Also prepared by esterification with the appropriate acid anhydride or acyl chloride in pyridine were methyl α-D-glucopyranoside tetraacetate and tetrabenzoate, maltose octabenzoate, and cellobiose octaacetate and octabenzoate. β-D-Glucopyranose pentaacetate and β-maltose octaacetate were prepared by esterification with acetic anhydride in the presence of sodium acetate[19]. β-D-Glucopyranose pentabenzoate was prepared by the method of Hudson *et al.*[7]. Methyl β-cellobioside heptaacetate and heptabenzoate were prepared from the corresponding hepta-*O*-acyl-α-cellobiosyl bromide[20]. Methyl β-D-glucopyranoside tetraacetate and tetrabenzoate and methyl β-maltoside heptaacetate were prepared by similar procedures[21,22]. Methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzoyl-α-D-glucopyranoside was prepared by benzoylation of methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside[23] in pyridine. All of the compounds had m.p. and $[\alpha]_D$ in agreement with the published values.

2,3-Di-*O*-acetyl-6-*O*-tritylamylose and 2,3-di-*O*-acetylamylose were prepared from 6-*O*-tritylamylose by a published procedure[24]. 2,3-Di-*O*-benzoyl-6-*O*-tritylamylose and 2,3-di-*O*-benzoylamylose were prepared similarly. *p*-Toluenesulfonylation of 2,3-di-*O*-acetylamylose in pyridine gave 2,3-di-*O*-acetyl-6-*O*-*p*-tolylsulfonylamylose[24]. 2,3-Di-*O*-acetyl-6-*O*-benzoylamylose and 6-*O*-acetyl-2,3-di-*O*-benzoylamylose were prepared by a similar procedure.

Also synthesized by acylation with the acyl chloride in pyridine were amylose tris(chlorodiphenylacetate), amylose tris(2,3-dimethylvalerate), and amylose tris-(*o*-chlorobenzoate). The first two are insoluble in chloroform, and the last was precipitated by methanol, so none of them could be used in these studies. None of the peracylated products showed any OH absorption in their i.r. spectra.

Methanolysis and reacetylation of amylose triacetate. — Amylose triacetate

(5 g) was dissolved in chloroform (375 ml) in a flask fitted with a reflux condenser. The flask was placed in a water bath at 50°, and 125 ml of 2M methanesulfonic acid in methanol was added. After the elapse of 2.25 h, 200 ml of pyridine was added to stop the reaction. After the mixture had been kept overnight in a refrigerator, pyridinium methanesulfonate was removed by filtration, and the filtrate was evaporated to dryness under diminished pressure. Pyridine (75 ml) was added to the residue, and the evaporation was repeated. The residue was dissolved in 75 ml of pyridine; 30 ml of acetic anhydride was added, and the mixture was heated for 1 h at 50° and kept overnight at ~25°. The mixture was then poured into ethanol, stirred in a blender. The precipitate was removed by filtration, washed with water, slurried with ethanol in a blender, and air-dried; yield 4.6 g. The i.r. spectrum of this product showed no OH absorption.

Determination of reaction products. — Methanolyses were effected in a flask by using the same temperature and concentrations of acid and ester as those used in the polarimetric studies. Aliquots for the determination of methyl acetate and methyl benzoate were removed at appropriate intervals of time and added to cold pyridine; samples were kept in a refrigerator and, after the formation of crystals of pyridine methanesulfonate (a few min), a portion of the solution lying above the crystals was subjected directly to g.l.c. on a Carbowax column. A column temperature of 70°, detector and injector temperatures of 200°, and a gas flow-rate of ~15 ml/min were used for the determination of methyl acetate. Under these conditions, all of the volatile components of the mixture (methyl acetate, methanol, chloroform, and pyridine) were separated. For the determination of methyl benzoate, a column temperature of 150°, injector and detector temperatures of 300°, and a gas flow-rate of ~30 ml/min were used, giving a good separation of methyl benzoate from the other components of the mixture.

Aliquots for the determination of free sugars and glycosides were neutralized with pyridine; crystalline pyridinium methanesulfonate was removed by filtration, and the solution was evaporated to dryness. Some samples were deacylated at this point[25]; others were dissolved directly in pyridine, per(trimethylsilyl)ated, and determined by g.l.c. according to the method of Brobst and Lott[26] on an SE-30 column operating isothermally at 160°.

A Varian Aerograph Model 204-B gas chromatograph equipped with a flame-ionization detector and a disc integrator was used for the g.l.c. analyses.

Incorporation of ^{14}C from methanol-^{14}C. — Methanolyses were conducted in a 50-ml flask fitted with a reflux condenser. A solution of amylose tripropionate in chloroform (15 ml) (see Methanolysis) and 2.5 ml of methanol-^{14}C (40 μCi; 7×10^6 counts/min/ml) were placed in the flask, and the mixture was heated in a water bath at 50°. Then 2.5 ml of 4M methanesulfonic acid in methanol was added, and the first sample was taken. One-ml aliquots were withdrawn at appropriate intervals of time, and poured into 0.5 ml of pyridine on a planchet. Samples were first air-dried, and then dried for 1 h under an infrared lamp before counting was performed.

Glycoproteins. — Pronase digestion of ovalbumin and glucoamylase I, followed by isolation of glycopeptides by gel filtration on Sephadex G-25, were conducted by published procedures[27].

Benzoylation of the main glycopeptide fractions (one from ovalbumin, two from glucoamylose) was performed as follows: ovalbumin glycopeptide (150 mg) was suspended in pyridine (2 ml) and benzoyl chloride (1 ml) was added. This mixture was heated for 0.5 h in a water bath at 60°. A second 2 ml of pyridine was then added, and the dark mixture was kept overnight at ~25°. The mixture was evaporated to dryness under diminished pressure, the residue was dissolved in 50 ml of chloroform, and the solution was washed 3 times with water, dried, and evaporated to dryness. This product was contaminated with some benzoic acid.

The benzoylated glycopeptide thus prepared was dissolved in 15 ml of chloroform in a 50-ml flask (equipped with a reflux condenser) placed in a water bath at 50°. Then 5 ml of 2M methanesulfonic acid in methanol was added. Samples (2 ml) were withdrawn after elapse of 20, 44, and 68 h, and each was added to 1 ml of pyridine. After removal of pyridinium methanesulfonate and concentration, the solutions were investigated chromatographically.

After 68 h, the concentration of acid was increased from 0.5 to ~1M by the addition of 2.5 ml of 4M methanesulfonic acid in methanol. Samples (2 ml) were withdrawn at 24 and 96 h after this addition of acid, and were treated as for the preceding aliquots.

NOTE ADDED IN PROOF (Received November 9th, 1970)

p-Toluenesulfonylation in the primary position has been used to stabilize polysaccharide linkages to acid-catalyzed hydrolysis for structural characterization[28].

ACKNOWLEDGMENTS

This study was supported by Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-9139(71) administered by the Northern Utilization Research and Development Division, Peoria, Illinois, whose support is gratefully acknowledged. Appreciation is also expressed to Dr. David R. Lineback, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, for a gift of glucoamylase I.

REFERENCES

1 J. N. BeMiller and R. E. Wing, *Carbohyd. Res.*, 6 (1968) 197.
2 R. E. Wing and J. N. BeMiller, *Carbohyd. Res.*, 10 (1969) 441.
3 R. W. Taft, Jr., *J. Amer. Chem. Soc.*, 74 (1952) 2729.
4 C. N. Riiber, *Ber.*, 57 (1924) 1797.
5 A. Nowakowski and Z. Mroczkowski, *Compt. Rend.*, 218 (1944) 461.
6 C. S. Hudson and J. K. Dale, *J. Amer. Chem. Soc.*, 37 (1915) 1264.
7 R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *J. Amer. Chem. Soc.*, 72 (1950) 2200.
8 D. J. Bell, *J. Chem. Soc.*, (1934) 1177.

9 P. A. LEVENE AND A. L. RAYMOND, *J. Biol. Chem.*, 97 (1932) 763.
10 H. BREDERECK, *Ber.*, 68 (1935) 2299.
11 P. BRIGL AND H. GRÜNER, *Ann.*, 495 (1932) 60.
12 D. HORTON AND J. H. LAUTERBACH, *J. Org. Chem.*, 34 (1969) 86.
13 R. U. LEMIEUX AND A. A. PAVIA, *Can. J. Chem.*, 46 (1968) 1453.
14 P. L. DURETTE AND D. HORTON, *Chem. Commun.*, (1969) 516.
15 J. N. BEMILLER AND E. E. ALLEN, JR., *J. Polymer Sci.*, A-1, 5 (1967) 2133.
16 R. S. THEOBALD, *J. Chem. Soc.*, (1961) 5359.
17 I. A. WOLFF, D. W. OLDS, AND G. E. HILBERT, *J. Amer. Chem. Soc.*, 73 (1951) 346.
18 J. W. MULLEN II AND E. PACSU, *Ind. Eng. Chem.*, 34 (1942) 1209.
19 A. P. N. FRANCHIMONT, *Ber.*, 12 (1879) 1940.
20 E. PACSU, *J. Amer. Chem. Soc.*, 52 (1930) 2571.
21 H. G. FLETCHER, JR., *Methods Carbohyd. Chem.*, 2 (1963) 226.
22 T. J. SCHOCH, E. J. WILSON, JR., AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 64 (1942) 2871.
23 B. HELFERICH, H. BREDERECK, AND A. SCHNEIDMÜLLER, *Ann.*, 458 (1927) 111.
24 T. R. INGLE AND R. L. WHISTLER, *Methods Carbohyd. Chem.*, 5 (1965) 411.
25 G. ZEMPLÉN, A. GERECS, AND I. HADÁCSY, *Ber.*, 69 (1936) 1827.
26 K. M. BROBST AND C. E. LOTT, *Cereal Chem.*, 43 (1966) 35.
27 A. P. FLETCHER, G. S. MARKS, R. D. MARSHALL, AND A. NEUBERGER, *Biochem. J.*, 87 (1963) 265.
28 D. A. REESE, N. G. RICHARDSON, N. J. WIGHT, AND E. HIRST, *Carbohyd. Res.*, 9 (1969) 451.