An Improved Synthesis of 6-Deoxy-Analogues of Cyclodextrins and Amylose

Further Interpretations of the Proton Magnetic Resonance Spectra of the Peracetates of Cyclodextrins and Amylose

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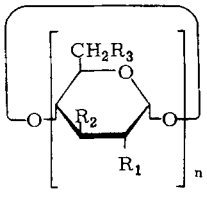
A convenient procedure for the synthesis of 6-deoxy-analogues of cyclodextrins and amylose was developed. Selective bromination of primary hydroxyl groups in these compounds, and subsequent reductive debromination of the 2,3-di-○-acetates lead to the corresponding 6-deoxy-derivatives after deacetylation. The conformation of the 2,3-di-○-acetates of 6-bromo-6-deoxy- and 6-deoxy-analogues of the dextrins and amylose was investigated by PMR-spectroscopy. Furthermore, the chemical shift changes due to the modification at the C-6 position was studied for the ring and acetyl-methyl protons.

(Summary see page 117; Zusammenfassung siehe Seite 117; Résumé à la page 117)

Introduction[1]

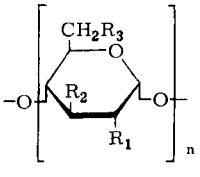
In the course of conformational studies on cyclodextrins and amylose in our laboratory [1, 2], it became desirable to accomplish preferential deoxygenation at the primary hydroxyl groups of all the glucopyranose residues of α-, β-, and γ-cyclodextrins (*1*, *2*, and *3*, respectively) and amylose (*19*). As for 6-deoxy-amylose (*21*), an outline of preparation through multi-step procedures was reported by *Bines* and *Whelan* [3], but with no experimental details. However, in our duplicate of their synthetic procedure, the products obtained were found to involve considerable amounts of unknown sugars other than 6-deoxy-D-glucose on acid hydrolysis.

Furthermore, in using 6-○-p-toluenesulfonate as the key intermediate in the synthesis of 6-deoxy-analogues (*7*, *8*, *9*, and *21*) of the three dextrins and amylose, some shortcomings were encountered; on tosylation of α-dextrin (*1*) or amylose (*19*) leading to a maximum substitution of the primary hydroxyl groups, it was demonstrated that not only introduction of tosyl groups [4] but also incorporation of chlorine into hydroxyl groups [5, 6] occur inevitably. On the other hand, selective tritylation of primary hydroxyl groups of the dextrins may lead to the formation of positional isomers [7] because full substitution of all the primary hydroxyl groups of the dextrin ring by trityl groups is impossible due to severe steric overlapping of the bulky substituents between adjacent glucose units [8].



1 n = 6, $R_1 = R_2 = R_3 = OH$
2 n = 7, $R_1 = R_2 = R_3 = OH$
3 n = 8, $R_1 = R_2 = R_3 = OH$

4 n = 6, $R_1 = R_2 = OH$, $R_3 = Br$
5 n = 7, $R_1 = R_2 = OH$, $R_3 = Br$
6 n = 8, $R_1 = R_2 = OH$, $R_3 = Br$
7 n = 6, $R_1 = R_2 = OH$, $R_3 = H$
8 n = 7, $R_1 = R_2 = OH$, $R_3 = H$
9 n = 8, $R_1 = R_2 = OH$, $R_3 = H$
10 n = 6, $R_1 = R_2 = OCOCH_3$, $R_3 = Br$
11 n = 7, $R_1 = R_2 = OCOCH_3$, $R_3 = Br$
12 n = 8, $R_1 = R_2 = OCOCH_3$, $R_3 = Br$
13 n = 6, $R_1 = R_2 = OCOCH_3$, $R_3 = H$
14 n = 7, $R_1 = R_2 = OCOCH_3$, $R_3 = H$
15 n = 8, $R_1 = R_2 = OCOCH_3$, $R_3 = H$
16 n = 6, $R_1 = R_2 = R_3 = OCOCH_3$
17 n = 7, $R_1 = R_2 = R_3 = OCOCH_3$
18 n = 8, $R_1 = R_2 = R_3 = OCOCH_3$



19 $R_1 = R_2 = R_3 = OH$
20 $R_1 = R_2 = OH$, $R_3 = Br$
21 $R_1 = R_2 = OH$, $R_3 = H$
22 $R_1 = R_2 = OCOCH_3$, $R_3 = Br$
23 $R_1 = R_2 = OCOCH_3$, $R_3 = H$
24 $R_1 = R_2 = R_3 = OCOCH_3$

A mixture of methanesulfonyl bromide and N,N-dimethylformamide (DMF) was used for the selective replacement of primary hydroxyl group in methyl α-D-glucopyranoside [9]. Utilizing this, we could successfully undertake the synthesis of 6-bromo-6-deoxy-analogues (*4*, *5*, *6*, and *20*) of the three dextrins and amylose, which are effectively used as good precursors to the 6-deoxy-analogues (*7*, *8*, *9*, and *21*). As for 6-halo-6-deoxy-amylose, during preparation of this manuscript, an analogous preparation of 6-chloro-6-deoxy-amylose [10] by use of the similar *Vilsmeier* type reagent, methanesulfonyl chloride-DMF [11] was reported.

In this paper, we describe the preparation of 6-bromo-6-deoxy-analogues of the three dextrins and amylose by use of the reagent, methanesulfonyl bromide-DMF, and their conversion into the corresponding 6-deoxy-analogues by

1 DMF = N,N-Dimethyl formamide; DMSO = Dimethyl sulfoxide; DS = Degree of substitution; glc = Gas liquid chromatography; tlc = Thin layer chromatography; pmr = Proton magnetic resonance.

reductive debromination with sodium borohydride in dimethyl sulfoxide (DMSO). We also report the proton magnetic resonance (pmr) spectra of the 2,3-di-○-acetates of 6-bromo-6-deoxy- and 6-deoxy-analogues obtained, compared with those of the peracetates (*16*, *17*, *18*, and *24*) of the three dextrins and amylose, for which some conformational problems have remained still unsolved [1, 12, 13].

Results and Discussion

The bromination procedure of the three dextrins (*1*, *2*, *3*) and amylose (*19*) was essentially the same as that used in the corresponding reaction of monosaccharide [9]. Treatment of the dextrin with 5 equiv. of methanesulfonyl bromide (based on glucose units) in DMF at 65 °C for 18 h gave a brominated, formylated product. After deformylation with sodium methoxide in methanol, 6-bromo-6-deoxy-dextrin was obtained as a white, water insoluble powder in good yield (95—98 %) having degree of substitution (DS) by bromine approximately 1.0. Attempts to brominate granular amylose with this reagent resulted in incomplete dissolution of amylose into DMF and gave a product containing very little bromine. Similar behavior has already been noted with the chlorination of amylose [10] or with the sulfation of cellulose [14]. Therefore, amylose was brought into a reactive form that the reagent could easily penetrate into the interior of the amylose particle. The granular amylose was recrystallized from aqueous solution saturated with n-butanol. The precipitate of n-butanol complex formed was thoroughly dehydrated by washing with ethanol and than washing with diethylether to give a fluffy form of amylose, with which the bromination reaction was markedly accelerated. This reactive form of amylose underwent bromination with 10 equiv. of methanesulfonyl bromide in DMF during 20 h at 65 °C. After destroying the ○-formate esters followed by dialysis, 6-bromo-6-deoxy-amylose (*20*) was isolated in 91 % yield as a white, water insoluble product whose DS by bromine was 0.92.

For converting the 6-bromo-6-deoxy-analogues (*4*, *5*, *6*, and *20*) into the corresponding 6-deoxy-analogues (*7*, *8*, *9*, and *21*), lithium aluminium hydride reduction could not be used because of the insolubility of the 6-bromo-6-deoxy-analogues in ether or tetrahydrofuran. All attempts to reduce the 2,3-di-○-acetyl-6-bromo-6-deoxy-analogues (*10*, *11*, *12*, and *22*) were unsuccessful; reducing agents tried were Raney nickel W6-hydrogen, 10 % Pd on charcol-hydrogen and Adams catalyst-hydrogen. Accordingly, we undertook the reductive debromination by using sodium borohydride in DMSO. The reduction of halogeno-derivatives with sodium borohydride in dipolar aprotic solvents [15] has not been widely used in the field of carbohydrate chemistry, but reductive dehalogenation of benzyl-2,3-di-○-acetyl-6-iodo-6-deoxy-4-○-tosyl-β-D-glucopyranoside with this reagent in DMSO has been recently reported [16]. Initially, when the free 6-bromo-6-deoxy-sugars (*4*, *5*, *6*, and *20*) were treated with this reagent, some difficulties were encountered. Namely, when heating at 70 °C was continued, the reaction mixture eventually set to gel. In order to avoid the unfavorable gelation, the 6-bromo-6-deoxy-analogues were converted into the corresponding 2,3-di-○-acetates (*10*, *11*, *12*, and *22*) with acetic anhydride and pyridine in the usual manner. With all the 2,3-di-○-acetates, the borohydride reaction was smoothly performed in a reasonable short period of 2 h at 70 °C. During this process, some acetyl groups were also removed so that the resulting product was fully re-acetylated. In this reduction, use of DMF in place of DMSO as solvent was also successful. Finally, deacetylation of the 2,3-di-○-acetates (*13*, *14*, *15*, and *23*) of 6-deoxy-analogues with sodium methoxide in methanol afforded the desired 6-deoxy-analogues (*7*, *8*. *9*, and *21*) of the three dextrins and amylose in good yields (89—95 %). On the contrary, parallel attempts to reduce the 6-chloro-6-deoxy-derivatives of the three dextrins and amylose by using sodium borohydride-DMSO under the same reaction conditions have so far been unsuccessful; the reduction of 6-chloro-6-deoxy-analogues prepared by treatment with a mixture of methanesulfonyl chloride and DMF [11] resulted in recovery of the starting materials. This was the reason of choice of the above bromo-deoxy-derivatives as the precursor for the synthesis of the deoxy-compounds.

Proof of the structures of the 6-deoxy-analogues (*7*, *8*, *9*, and *21*) an evaluation of the extent of the selective deoxygenation at the primary hydroxyls in these compounds were carried out by identification of the products obtained by complete methanolysis. Thinlayer chromatography (tlc) proved the methanolizates to be composed of one minor and one major component. The latter showed the mobility identical with that of authentic methyl 6-deoxy-α- and β-D-glucopyranosides. Gas liquid chromatography (glc) greately simplified the identification. The components of the methanolizates were converted into their trimethylsilyl-ether before injection. By co-injection of reference compound, i.e., authentic methyl 6-deoxy-α- and β-D-glucopyranoside, with the methanolizate, the presence of methyl 6-deoxy-α- and β-D-glucopyranosides in the methanolizates was established from their retention times. Glc analysis also showed that compounds *7*, *8*, *9*, and *21* are composed of 95, 97, 94, and 92 % 6-deoxy-D-glucose, respectively. On a relatively large scale, purification of the methanolizate on a silica gel column afforded a chromatographically pure product in a good yield, which was partially crystallized on standing in the air. This product was identified to be a mixture of methyl 6-deoxy-α- and β-D-glucopyranosides in comparison of the pmr spectrum with that of authentic methyl 6-deoxy-α- or β-D-glucopyranoside.

These results indicate that the selective bromination by use of methanesulfonyl bromide-DMF satisfactorily occured at the expected position of the glucopyranose units of *1*, *2*, *3*, and *19*, and that the subsequent reductive debromination was completely performed with sodium borohydride in DMSO. Considering the difficulties experienced with these compounds, the purity and yields obtained are to be said satisfactory. The above deoxygenation procedure provides improvement over current sulfonylation procedure [5, 6] in the selectivity, yield and its simplicity in the reaction procedure.

It was shown that the analogous chlorination of methyl α-D-glucopyranoside by a mixture of methanesulfonyl chloride and DMF under drastic reaction conditions gives, in addition to the expected methyl 6-chloro-6-deoxy-α-D-

glucopyranoside, methyl 4,6-di-chloro-4,6-di-deoxy-α-D-galactopyranoside, the secondary hydroxyl groups at C-2 and C-3 remaining unaffected [17]. This suggests that, in the series of the sugars having α-anomeric configuration, the reactivity of this reagent to the secondary hydroxyl groups at C-2 and C-3 was considerably less than that to the secondary hydroxyl group at C-4. From the reactivity sequence for the secondary hydroxyl groups, the observed high-selectivity of the primary hydroxyl groups of *1*, *2*, *3*, and *19* toward the bromination may be quite reasonable, since these compounds, whose most reactive secondary hydroxyl groups at C-4 are blocked by α, 1 – 4 glycosidic linkage, were subjected merely to mild bromination.

Having achieved selective substitution at the primary hydroxyl groups, it is interesting to compare the pmr spectra of the 2,3-di-○-acetates of 6-bromo-6-deoxy- and 6-deoxy-analogues of the three dextrins and amylose with those of the peracetates (*16*, *17*, *18*, and *23*).

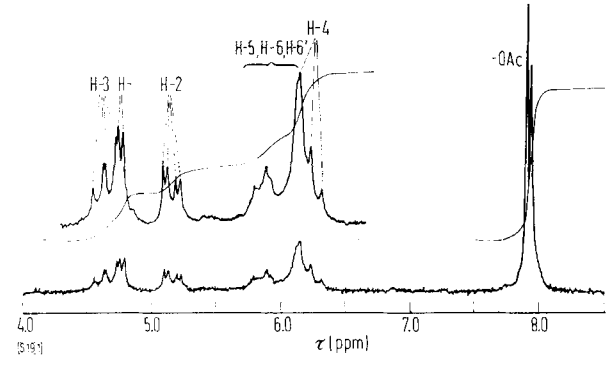


Figure 1. Pmr spectrum of 2,3-di-○-acetyl-6-bromo-6-deoxy-β-cyclodextrin in $CDCl_3$ at 100 MHz.

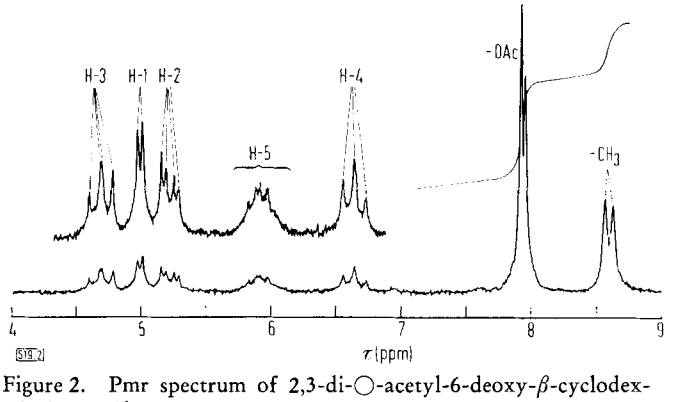


Figure 2. Pmr spectrum of 2,3-di-○-acetyl-6-deoxy-β-cyclodextrin in $CDCl_3$ at 100 MHz.

The pmr spectra of *11* and *14* are shown in Figures 1 and 2, respectively. The partial pmr spectra of *22* and *23* are also shown in Figures 3 and 4, respectively. In Table 1 are given the pmr data for the ring and acetyl-methyl protons of *10*, *11*, *12*, and *22* and for the ring, acetyl-methyl and methyl protons of *13*, *14*, *15*, and *23*.

Concerning the pmr spectra of *16*, *17*, and *18*, details of assignments of the signals for the individual ring protons have been reported by *Casu* et al. [12] and by the present authors [1], independently. The pmr data for the ring and acetyl-methyl protons of *16*, *17*, and *18* are also shown in Table 1 for comparison. In the present study, assignments for the signals to individual ring protons were based on previous assignments for *16*, *17*, and *18* [1]. Most of signals approach a first-order pattern and allow an approximate evaluation of the coupling constants directly from the signal splitting. However, the chemical shift of H-5, H-6, and H-6′ can not be obtained accurately from our spectra.

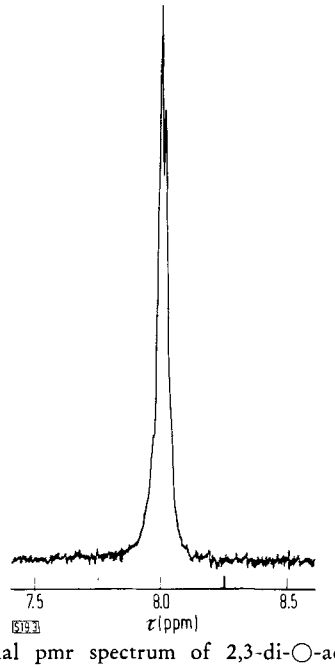


Figure 3. Partial pmr spectrum of 2,3-di-○-acetyl-6-bromo-6-deoxy-amylose in $CDCl_3$ at 100 MHz.

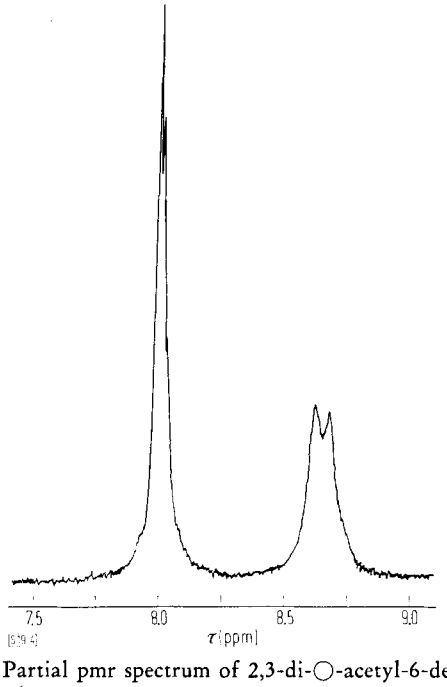


Figure 4. Partial pmr spectrum of 2,3-di-○-acetyl-6-deoxy-amylose in $CDCl_3$ at 100 MHz.

To estimate the magnitude of the chemical shift changes due to a given substitution at C-6, the pmr spectra of β-dextrin derivatives, *11* and *14* were compared with that of the peracetate (*17*) of β-dextrin.

The comparison of the spectra of *11* and *17* or of *14* and *17* showed interesting deshielding or shielding effect on the ring protons by modification at C-6. On the bromination at

Table 1. Chemical Shifts (τ) and First-Order Coupling Constants (Hz, given in parentheses) of the Ring, Acetyl-Methyl and/or Methyl Protons of 2,3-Di-O-acetyl-6-bromo-6-deoxy-analogues, 2,3-Di-O-acetyl-6-deoxy-analogues and 2,3,6-Tri-O-acetates of α-, β-, and γ-Cyclodextrins and Amylose in $CDCl_3$ at 100 MHz.

Compound	H-1 doublet	H-2 double doublet	H-3 double doublet	H-4 triplet	H-5 or H-5, H-6, H-6′ complex multiplet	OAc singlet	CH_3 doublet
10	4.82 (3.5)	5.17 (3.5, 10.5)	4.49 (8.5, 10.0)	6.15 (9.0)	5.70–6.13	7.94, 7.95	
11	4.77 (3.5)	5.16 (3.5, 10.0)	4.64 (8.0, 10.0)	6.23 (9.0)	5.80–6.15	7.91, 7.94	
12	4.71 (3.5)	5.22 (3.5, 10.0)	4.64 (9.0, 10.0)	6.23 (9.5)	5.88–6.14	7.91, 7.93	
22						8.00, 8.01	
13	4.99 (4.0)	5.21 (3.5, 10.0)	4.56 (8.5, 10.5)	6.53 (8.0)	5.85–6.01	7.95, 7.96	8.60 (6.5)
14	5.00 (3.5)	5.23 (3.5, 10.0)	4.71 (8.5, 10.0)	6.65 (9.0)	5.84–5.99	7.92, 7.95	8.60 (6.0)
15	4.96 (3.5)	5.26 (3.5, 10.0)	4.69 (8.5, 10.0)	6.65 (9.0)	5.80–6.18	7.92, 7.93	8.62 (6.0)
23						8.01, 8.02	8.65 (6.0)
16[1]	4.92 (3.0)	5.21 (3.5, 9.5)	4.41 (8.0, 9.5)	6.19 (9.5)	5.43–5.95	7.80, 7.92, 7.93	
17[1]	4.90 (3.0)	5.20 (3.5, 9.5)	4.69 (8.0, 9.5)	6.28 (9.5)	5.30–5.95	7.88, 7.90, 7.94	
18[1]	4.86 (3.0)	5.25 (3.5, 9.5)	4.64 (8.0, 9.5)	6.29 (9.5)	5.38–6.02	7.88, 7.90, 7.92	
24[2]						7.84, 8.02, 8.06	

1) Data taken from ref. [1]; 2) Data taken from ref. [12].

C-6, the resonance of the ring protons of *11* are uniformly shifted downfield compared with the signals for *17*. The difference in chemical shifts between the H-2, H-3, and H-4 resonances is small ($< \Delta\tau - 0.05$ ppm) whereas the difference in chemical shift between the H-1 resonances relatively increases ($\Delta\tau - 0.13$ ppm). On the contrary, on the deoxygenation at C-6, the uniform upfield shift is seen for the ring protons of *14* compared with the signals for *17* and the largest shift for H-4 signal. The difference in the chemical shifts between the H-2 and H-3 resonances is small ($< \Delta\tau + 0.04$ ppm), but the difference between the H-1 and H-4 resonances increases; thus $\Delta\tau$, H-1, +0.10 ppm; and H-4, +0.37 ppm. Essentially the similar trend is observed with the pmr spectra of the derivatives of α- and γ-dextrins. However, a little exception was encountered for the chemical shift differences between the H-3 resonances of *10* and *13*. Compared with the H-3 signal of *16*, the signal of *10* is shifted upfield by +0.08 ppm, i.e., a positive shift. The H-3 resonance of *13* was shifted upfield by +0.15 ppm, being a relatively large shift compared with those observed for the H-3 signal of *14* and *15*.

Provided that the deshielding effect observed with the H-1 resonance of *11* depends merely on the electron-withdrawing effect of the halogen at C-6, the H-4 proton should be simultaneously deshielded to the same extent as observed with the H-1. However, this is not indeed the case. Inspection of molecular models offers an explanation for this deshielding of the H-1. The dextrins are torus shaped-molecules with the glucopyranose residues in substantially undistorted C-1 conformation [1, 12]. One of the open ends of the cavity is surrounded by the primary hydroxyl groups situated at C-6 of the glucopyranoses and the other end by the secondary hydroxyl groups at C-2 and C-3 [18]. The substitution of the secondary hydroxyl groups at C-2 and C-3 by acetyl groups and the replacement of the primary hydroxyl groups at C-6 by bromine may give rise to severe sterically overcrowding of the acetyl groups situated on one "secondary" side of the dextrin. The departure from the steric restraints imposed by the acetyl groups on "secondary" side may be accomplished by rotation of the glucose units about the glycosidic C-1-O and C-4-O bonds, thus leading to large deviations from the symmetrical alignment of the monomeric units in the dextrin ring and further accentuating the "V" shaped nature [19] of the dextrin cavity. In this arrangement of the chain conformation, it seems likely that H-1 of *11* may be subjected to *van der Waal*'s shift [20] due to the pronounced close proximity of H-1 and the bromine at C-6 on the adjacent glucose unit, causing a downfield shift of the H-1 resonance of *11*.

On the other hand, molecular model of *14* reveals that such *van der Waal*'s shift experienced by H-1 of *11* is ignored in *14*, because of the relatively long distance between H-1 and methyl groups at C-6 on the adjacent unit, so that increase in the shielding of H-1 and H-4 of *14* may be considered caused only by the replacement of the acetyl group at C-6 of *17* by hydrogen. This shows that the acetyl group at C-6 of *17* does contribute to significant alteration of the local magnetic field at H-4 on the same glucose unit and at H-1 on the contiguous unit.

The noticeable upfield shifts observed for H-3 of *10* and *13* may be most likely understood from decrease in the deshielding of the axial hydrogen atom (H-3) by an opposing glycosidic oxygen atom in axial orientation. In the previous paper [1], the lowest field resonance for H-3 of *16* has been explained in terms of the deshielding effect of the axially opposed glycosidic oxygen atom at C-1 in syndiaxial relation to H-3, besides the deshielding effect due to the acetyl group at C-3. It might be expected that the deshielding effect of the lone pairs of the glycosidic oxygen atom on H-3 becomes the greatest, when the glucose units of the dextrin ring will approach a symmetrical alignment. From the consideration of molecular models, it may be inferred that the greatest deviations from the symmetrical alignment of the glucose units in the dextrin ring should exist in the smallest members (*10* and *13*) of the derivatives of the three dextrins, whose steric requirements are the most intense. As a consequence of specific modification of the acetyl groups at the C-6 position, the orientation of the lone pair orbitals of the glycosidic oxygen atom of *10* and *13* may be neither coplaner nor diaxial relation to H-3, thus decreasing the extent of the deshielding effect of the glycosidic oxygen on H-3.

In connection with the conformation of *16* and *17*, *Casu* et al. [12] have claimed that the acetyl groups appear to effect essentially only the protons on the carbon atoms to which they are attached, i.e., C-2, C-3, and C-6, but have little influence on the chemical shift of H-1 and H-4, and that the upfield shift of the signals for H-1 and H-4 of *16* and *17* is attributed to a definite effect of diamagnetic anisotropy of glycosidic C-4-O and C-1-O bonds on H-1 and H-4 on contiguous units, respectively, which is caused by restricted rotation of the monomeric units about the glycosidic bonds due to the cyclic nature of the dextrins [12]. However, the experimental results obtained above demonstrate that the acetyl group at C-6 affects not only the H-1 resonance on contiguous unit but also the H-4 resonance on the same unit. Furthermore, it is obvious that the contribution of the diamagnetic anisotropy of C-1-O and C-4-O bonds to the chemical shift for H-4 and H-1 on contiguous units, respectively, is by far small as compared with the magnetic anisotropic effect on H-1 and H-4 due to the acetyl group at C-6.

With the signal assignment of the acetyl-methyl protons of the peracetates (*16* and *17*) of the dextrins, *Casu* et al. tentatively assigned the most downfield acetyl-methyl signal of *16* and *17* to the acetyl group at C-3 on the basis of solvent effect on acetyl-methyl proton signal in reference to some mono- and oligo-saccharides [12]. However, as shown in Figures 1 and 2, the most downfield acetyl-methyl signal of *17* disappeared on the bromination or deoxygenation of the primary hydroxyl groups at C-6. In these derivatives, the shielding or deshielding effect due to the substituents at C-6 on the acetyl-methyl signals at C-2 and C-3 in the same glucose unit is almost disregarded because of the long distance between the substituent and these acetyl groups. Consequently, the results obtained here provide direct evidence that the most downfield acetyl-methyl signal of *16*, *17*, and *18* can be assigned to the acetyl group at C-6.

It was pointed out [12, 13] that peracetylated amylose (*24*) shows two acetyl-methyl signals in the equatorial region [21] and one acetyl-methyl signal in the axial region [21]. However, a controversy exists over the assignment of the acetyl-methyl signal of the peracetate (*24*). In one report [13], the most downfield signal appeared in the axial region was assigned to the acetyl group at C-6, whereas, in another [12] the most upfield signal to this. The partial pmr spectra of *22* and *23* shown in Figures 3 and 4, respectively, did not show any signals in the axial region, suggesting that the acetyl-methyl signal of the peracetate (*24*) appearing in the axial region is of the acetyl group at C-6. This finding is well consistent with that obtained for *16*, *17*, and *18*. It is noteworthy that primary acetyl-methyl signals in the equatorial orientation at C-6 in glucopyranose rings of *16*, *17*, *18*, and *24* resonate at a field that is typical for axially oriented groups in monosaccharides [21, 22, 23]. This fact demonstrates that the correlation of the conformations of the glucopyranose ring and the chemical shift of the acetyl-methyl signal which is worked out for monosaccharides [21, 22, 23] are no longer valid for macromolecular structures, where possible nonbonded and dipolar interactions between substituents on adjacent glucose units may strongly affect the backbone chain conformation of the macrocyclic or polymeric carbohydrate.

Experimental

Materials

All the reagents used were of commercially available. DMF and DMSO were dried over calcium hydride, distilled and stored over Drielite. All other solvents were once distilled. Methanesulfonyl bromide was prepared in the similar method described by *Sieber* [24]. The cyclodextrins used were the same as those previously described [25]. Amylose was provided by Nichiden Kagaku Co. Ltd., Osaka. The conversion of this granular amylose into a reactive form was carried out as follows. The amylose (20 g) was dissolved into 1N-aqueous solution (750 ml) of sodium hydroxide by vigorous stirring and was stirred until clear solution was obtained. Undispersed materials were removed by suction-filtration and the pH of the solution was adjusted to 5.7 by adding 1N-hydrochloric acid solution, and the solution was then diluted with distilled water so as to obtain 1 % amylose solution. The resulting solution was immediately heated to boiling and n-butanol (200 ml) was added and was shaken vigorously. The container was tightly stoppered to prevent the vaporization of n-butanol and was placed in a Dewar flask filled with boiling water, and the temperature was gradually lowered to room temperature. The precipitate formed was recovered by centrifugation at 3000 rpm and was transferred into ethanol (500 ml) in a closed vessel and was stirred for 10 h. The precipitate was filtered off through a glass filter and was again dehydrated with ethanol (500 ml) by stirring. This dehydration process was repeated with ethanol (500 ml × 2) and the precipitates were finally stirred in ether (500 ml × 2), filtered off by suction and then dried *in vacuo* at 80 °C over phosphorus pentoxide to give a fluffy form of amylose (18 g). The final thorough stirring with ether is absolutely necessary for the preparation of the reactive form of amylose capable of swelling in DMF.

General Procedure

Melting points were determined with a Yanagimoto micro-melting point measuring apparatus and are uncorrected. Optical rotations were measured with a Ohyo Denki polarimeter, Model MP-1T, in a 1 dm tube. Pmr spectra were taken at 100 MHz with a Varian HA-100 spectrometer using tetramethylsilane as an internal standard. Chemical shifts are given on the τ scale and the apparent coupling constants were measured directly from the signal splitting. Shimadzu Gas-Chromato, Model GC-3AF, was employed for glc. The column was 3 m length of 3 mm in internal diameter copper tubing, packed with 5 % SE-30 on Shimalite W. Operation condition was isothermal at 170 °C, with nitrogen gas as the carrier gas, and flame ionization detection. Tlc was performed on silica gel G (Merck) and the plate prepared was activated at 110 °C before use. Solvent system used was ethylacetate : ethanol : H_2O (45 : 5 : 3 v/v) and 20 % aqueous sulfuric acid spray followed by heating at 120 °C were used to detect the methanolizates on tlc plates. Column chromatography was carried out by use of silica gel (Kanto Kagaku Co., 60—80 mesh). Developing solvent used was the same as that used for tlc. Unless otherwise stated, solutions were concentrated or evaporated at

bath temperature not exceeding 45 °C under reduced pressure and drying of the materials obtained was carried out over phosphorus pentoxide at 80 °C *in vacuo*.

6-Bromo-6-deoxy-analogues (4, 5, 6, and 20)

Methanesulfonyl bromide (5 equiv. for *1*, *2*, and *3*, and 10 equiv. for *19*, based on the constituent glucose units) was added over a period of 30 min to a stirred solution of the sugars in anhydrous DMF (1 g/16 ml for *1*, *2*, and *3*, and 1 g/40 ml for *19*) maintained at 65 °C. After stirring at this temperature for 16 h for *1*, *2*, and *3*, and 20 h for *19*, the resulting yellow solution was concentrated and coevaporated with toluene to give a pale-brown thin syrup, which was dissolved in methanol and was neutralized with 3 M sodium methoxide in methanol to destroy the ◯-formate esters [9]. The resulting mixture was then added into ice-water and was vigorously stirred. The supernatant was removed by decantation and the precipitate was filtered off on a Büchner funnel, exhaustively washed with cold water and sucked dry. The solid thus obtained was dried to give 6-bromo-6-deoxy-analogues *4*, *5*, and *6* in 95, 98, and 95 % yield, respectively, as white, water insoluble powder. In the isolation of *20*, the mixture obtained by treatment with sodium methoxide was dialized against running water for 48 h followed by distilled water for 20 h, and then the non-dialyzable product was freeze-dried to afford *20* in 91 % yield as a white material. These derivatives gave a strong positive *Beilstein* test for halogen and were insoluble in the common organic solvents but was soluble in pyridine, DMF and DMSO.

4 mp 195–196 °C (decomp), $[\alpha]_D^{25}$ + 91° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.05}Br_{0.95}]_6$: C 32.53, H 4.01, Br 34.07. Found: C 32.59, H 4.01, Br 34.77.

5 mp 205–206 °C (decomp), $[\alpha]_D^{25}$ + 98° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.03}Br_{0.97}]_7$: C 32.29, H 4.09, Br 33.84. Found: C 32.99, H 4.06, Br 33.09.

6 mp 201–202 °C (decomp), $[\alpha]_D^{25}$ + 103° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.06}Br_{0.94}]_8$: C 32.56, H 4.13, Br 33.95. Found: C 32.47, H 4.16, Br 33.70.

20 $[\alpha]_D^{25}$ + 130° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.08}Br_{0.92}]_n$: C 32.74, H 4.17, Br 33.41. Found: C 33.01, H 4.15, Br 33.56.

2,3-Di-◯-acetyl-6-bromo-6-deoxy-analogues (10, 11, 12, and *22)*

To each solution of 6-bromo-6-deoxy-derivatives (1 g) in dry pyridine (15 ml) was added acetic anhydride (10 ml) at room temperature with stirring. After standing for 30 h with occasional shaking, the viscous solution was poured in a thin stream into 5 volumes of ice-water with efficient stirring. The precipitate separated was corrected on a filter paper, washed well with cold water, filtered off and dried to give a white solid, which was recrystallized from ethanol.

10 mp 179–181 °C, $[\alpha]_D^{25}$ + 89° (c 1, $CHCl_3$).

11 mp 193–195 °C, $[\alpha]_D^{25}$ + 100° (c 1, $CHCl_3$).

12 mp 196–199 °C, $[\alpha]_D^{25}$ + 112° (c 1, $CHCl_3$).

22 $[\alpha]_D^{25}$ + 148° (c 1, $CHCl_3$).

2,3-Di-◯-acetyl-6-deoxy-analogues (13, 14, 15, and 23) and 6-Deoxy-analogues (7, 8, 9, and 21)

Sodium borohydride (5 equiv. based on the constituent 2,3-di-◯-acetyl-6-bromo-6-deoxy-glucose units) was added in DMSO (60 ml) in one portion and the temperature was raised to 70 °C. After the sodium borohydride had dissolved, the 2,3-di-◯-acetate (*10, 11, 12* and *22*) of 6-bromo-6-deoxy-analogues (2 g) in DMSO (20 ml) was slowly added to the borohydride solution with stirring and the mixture was further stirred for 2 h at 70 °C with exclusion of moisture. Rapid gas evolution was observed at the early stage of the reaction. Most of DMSO was removed from the reaction mixture by distillation *in vacuo* below 50 °C and the resulting solid was carefully poured portionwise into cold 2 % acetic acid solution (200 ml) containing a trace of n-octanol to destroy excess sodium borohydride. After effervescence had ceased, the solution was cooled and extracted three times with chloroform (70 ml × 3) to separate the product from inorganic salts. The combined extracts were washed with ice-water until neutralized, dried over sodium sulfate and concentrated. The residue was re-acetylated with acetic anhydride in pyridine in the usual manner to give the 2,3-di-◯-acetyl-6-deoxy-analogues *13, 14, 15,* and *23* in 91, 95, 89, and 92 % yields, respectively, which were recrystallized from iso-propanol to give a white powder. The *Beilstein* test for these compounds was negative, indicating that no bromine remained.

13 mp 160–163 °C, $[\alpha]_D^{25}$ + 89° (c 1, $CHCl_3$).

14 mp 193–195 °C, $[\alpha]_D^{25}$ + 100 °C (c 1, $CHCl_3$).

15 mp 196–198 °C, $[\alpha]_D^{25}$ + 112° (c 1, $CHCl_3$).

23 $[\alpha]_D^{25}$ + 148° (c 1, $CHCl_3$).

These 2,3-di-◯-acetates were deacetylated with sodium methoxide in methanol in the conventional manner to afford 6-deoxy-analogues *7, 8, 9,* and *21* in almost quantitative yields. The compounds *7* and *8* gave brown and pale-yellow colorations, respectively, on tlc plate by spraying iodine-methanol solution, but no color development was observed for *9*. On reaction with aqueous iodine-iodide solution, the compound *21* did produce no blue color characteristic of unmodified amylose.

7 $[\alpha]_D^{25}$ + 103° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.05}H_{0.95}]_6$: C 49.03, H 6.87. Found: C 49.15, H 6.89.

8 $[\alpha]_D^{25}$ + 112° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.03}H_{0.97}]_7$: C 49.14, H 6.89. Found: C 49.08, H 6.80.

9 $[\alpha]_D^{25}$ + 120° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.06}H_{0.94}]_8$: C 48.96, H 6.84. Found: C 49.31, H 6.34.

21 $[\alpha]_D^{25}$ + 148° (c 1, pyridine). Anal. Calcd. for $[C_6H_9O_4(OH)_{0.08}H_{0.92}]_n$: C 48.87, H 6.85. Found: C 48.62, H 6.81.

Identification of the Methanolysis Products Obtained from 7, 8, 9, and *21*

A 100 mg portion of *7, 8, 9,* and *21* in 10 ml of 1 % methanolic hydrogen chloride was refluxed for 20 h under protection from moisture. After cooling, the solution was neutralized with Amberlite IRA-410 (CO_3^-) resin and evaporated to a syrup. Tlc of the methanolizates then disclosed

the products to be composed of two kinds of monoglycoside with R_f values of 0.54 (major spot) and 0.70 (minor spot), respectively; the relative intensities were consistent with those obtained by glc analysis mentioned below. The R_f value of major spot was in good agreement with that of authentic methyl 6-deoxy-α- and β-D-glucopyranosides and the spot was visualized as a intense yellow spot characteristic for the color-development of the deoxy-sugars with detection by spraying of sulfuric acid solution and then heating.

A solution of each methanolizate (20—30 mg) in pyridine (1 ml) was trimethylsilylated with hexamethyldisilazane (0.5 ml) and trimethylchlorosilane (0.25 ml). A reaction period for 20 h was used to ensure complete trimethylsilylation. The co-injection of the trimethylsilylated methanolizates with authentic sample, a mixture of trimethylsilylated methyl 6-deoxy-α- and β-D-glucopyranosides, showed the presence of large amounts of the component in the methanolizates, having the same retention time equal to those for the authentic samples. Comparison of the peak areas under the curves for the methanolizates and for the standard sample showed that the methanolizates obtained from *7*, *8*, *9*, and *21* are composed of 95, 97, 94, and 92 % methyl 6-deoxy-α- and β-D-glucopyranosides, respectively, implying that the DS values of 0.95, 0.97, 0.94, and 0.92 for *7*, *8*, *9*, and *21*, respectively.

Compound *8* (3 g) was methanolized in the same way as described above and the products obtained were chromatographed. After 1800 ml of the eluate had been passed through the column, every fraction (18 ml) was tested by tlc. The substance with R_f value of 0.54 appeared in the fraction numbers 45—65; these fractions were combined and evaporated to dryness, giving a syrup which was partially crystallized spontaneously on standing in the air (yield, 2.8 g), the structure of which was identified by pmr spectroscopy in DMSO-d_6, comparing the pmr data with those obtained for the authentic methyl 6-deoxy-α- or β-D-glucopyranoside:

α-anomer; mp 96—98 °C, $[\alpha]_D^{25}$ + 158° (c 1, water), pmr data (DMSO-d_6): 5.52 (3 H, broad, OH), 5.52 (1 H, doublet, H-1, J = 3.1 Hz), 6.71 (1 H, singlet, OCH_3), 8.89 (3 H, doublet, C—CH_3, J = 6.2 Hz).

β-anomer; mp 129—130 °C, $[\alpha]_D^{25}$ — 47° (c 1, water), pmr data (DMSO-d_6): 5.05 (3 H, broad, OH), 5.97 (1 H, doublet, H-1, J = 7.3 Hz), 6.60 (3 H, singlet, OCH_3), 8.86 (3 H, doublet, C—CH_3, J = 6.2 Hz).

The pmr data of the partially crystalline product isolated by column chromatography are well consistent with those of the above authentic α- or β-anomer in comparison of the chemical shifts and coupling constants. The structure of the minor by-product with R_f value of 0.70 on tlc could not be established because of its low yield.

Summary

A convenient synthetic procedure of 6-deoxy-analogues of α-, β-, and γ-cyclodextrins and amylose was developed. Selective bromination of all the primary hydroxyl groups of the starting compounds by using a mixture of methanesulfonyl bromide and dimethylformamide gave the corresponding 6-bromo-6-deoxy-analogues in good yields, with degree of substitution of approximately 1.0. Subsequent reductive debromination of the 2,3-di-○-acetates of 6-bromo-6-deoxy-derivatives was successfully accomplished with sodium borohydride in dimethyl sulfoxide to afford the corresponding 2,3-di-○-acetates of 6-deoxy-analogues in good yields, which were deacetylated to the expected 6-deoxy-derivatives. The purity and structures of the compounds thus obtained were confirmed by identification of methanolysis products of these compounds; structural elucidation of the methanolizates was carried out by means of glc, tlc, and pmr spectroscopy. It was found that the 6-deoxy-derivatives of α-, β-, and γ-dextrins and amylose are composed of 95, 97, 94, and 92 % 6-deoxy-D-glucose, respectively. The conformation of the 2,3-di-○-acetates of 6-bromo-6-deoxy- and 6-deoxy-analogues of the three dextrins and amylose was investigated by pmr spectroscopy, comparing their pmr spectra with those of the 2,3,6-tri-○-acetates. The chemical shift changes due to the modification at the C-6 position was also studied for the ring and acetyl-methyl protons and it was demonstrated that, in the 2,3,6-tri-○-acetates of the three dextrins, the acetyl group at C-6 contributes to significant alteration of the local magnetic field at H-4 on the same glucose unit and at H-1 on the contiguous unit. An acetyl-methyl signal appearing in the lowest field among the three acetyl-methyl signals in the pmr spectra of the 2,3,6-tri-○-acetates of the three dextrins and amylose could be assigned to the acetyl group at C-6.

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Zusammenfassung

Verbesserte Methode zur Synthese von 6-Deoxy-Analoga von Cyclodextrinen und Amylose. Weitere Deutungen der PMR-Spektren der Peracetate von Cyclodextrinen und Amylose. *Es wurde ein geeignetes Syntheseverfahren für 6-Deoxy-Analoga der Cyclodextrine und der Amylose entwickelt. Die selektive Bromierung der primären Hydroxylgruppen in diesen Verbindungen und die nachfolgende reduktive Debromierung der 2,3-Di-○-acetate führte nach Deacetylierung zu den entsprechenden 6-Deoxy-derivaten. Die Konformation der 2,3-Di-○-acetate der 6-Brom-6-deoxy- und 6-Deoxy-analoga der Dextrine und der Amylose wurde durch PMR-Spektroskopie ermittelt. Weiterhin wurden die auf die Modifikation in der C-6-Stellung zurückzuführenden chemischen Lageänderungen des Ring- und des Acetyl-methyl-protons untersucht.*

Résumé

Synthèse améliorée des 6-déoxy-analogues des cyclodextrines et de l'amylose. Interprétations plus étendues des spectres RMN des peracétates de cycodextrines et d'amylose. *Une méthode appropriée pour la synthèse des 6-déoxy-analogues des cyclodextrines et de l'amylose a été mise au point. Une bromation sélective des groupements hydroxyls primaires dans ces constituants, suivie d'une débromation des 2,3-di-○-acétates conduit à l'obtention des 6-déoxy-dérivés correspondants après déacétylation. La conformation des 2,3-di-○-acétates du 6-bromo-6-déoxy- et des 6-*

déoxy-analogues des dextrines et de l'amylose a été étudiée par la spectroscopie R M N. De plus, les variations du déplacement du spectre d'origine chimique, dues à la modification de la molécule en C-6 ont été étudiées dans le cas du proton du cycle et de celui du groupement acétyl-méthyl.

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Fractionation of Corn Syrup

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***Corn syrup** was fractionated on a cellulose support. An ethanol-water gradient elution was used. Carbohydrates were determined quantitatively by the phenol-sulfuric acid method. A normal 43 D.E. corn syrup gave 20 peaks and a materials balance of 92 %. A high maltose corn syrup gave 18 peaks and a 99—100 % materials balance.*

(Zusammenfassung siehe Seite 122; Résumé à la page 122)

Introduction

The carbohydrate composition of corn syrup has been investigated by many workers using such diverse techniques as: thin layer chromatography (TLC) [1, 2], gas liquid chromatography (GLC) [3], gel permeation chromatography (GPC) [4], and chromatography on cellulose [5, 6]. In addition the separation of oligosaccharides from a xylan was carried out on ion-exchange resins [7].

The corn syrup fractionation described in this paper was carried out by liquid chromatography on a cellulose support. Gradient elution was used (ethanol-water) and the collected fractions were examined for carbohydrates by the Phenol-Sulfuric Acid Method [8]. The homogeneity of the first seven peaks was determined by thin layer chromatography [9]. The quantitative amount of carbohydrates in each peak was determined with the aid of the Phenol-Sulfuric Acid Method and a standard calibration curve. Two types of corn syrups were examined: a normal 43 D.E. corn syrup and a high maltose corn syrup. Twenty peaks were obtained for the normal corn syrup and eighteen peaks for the high maltose corn syrup. The quantitative amount of each of the first four components in the corn syrups (Glucose to Maltotetraose) was determined by GLC [10] and compared to the results obtained by column chromatography. In addition a materials balance was carried out. For the normal corn syrup the carbohydrate recovery amounted to 92 % while for the high maltose corn syrup the recovery was 99—100 %.

Experimental

Cellulose preparation: The material (Whatman Cellulose Powder CC-31) was suspended in distilled water, stirred manually and left for 30—60 min at R.T. The mixture was then filtered on a Buchner funnel and the filter cake resuspended in water and the process repeated. After four or five washings a one ml aliquot of the wash water was tested with Phenol-H_2SO_4. If the test was positive the washing