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# Chemical Synthesis of Polysaccharides. VIII. Synthesis and Enzymatic Hydrolysis of (1→6)-α-Linked Heteropolysaccharides Consisting of D-Glucose and 2,3,4-Trideoxy-DL-*glycero*-hexopyranose Units

*Dedicated to the Memory of the late Professor Ichiro Sakurada*

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ABSTRACT: (1→6)-α-Linked heteropolysaccharides composed of D-glucose and 2,3,4-trideoxy-DL-*glycero*-hexopyranose units were synthesized by cationic ring-opening copolymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucose (TBLG) with *rac*-4(e)-bromo-6,8-dioxabicyclo[3.2.1]octane (BrDBO) followed by reductive debromination and subsequent debenzylation. The heteropolysaccharides (mole fraction of D-glucose unit, 0.34—0.90) were soluble in water and dimethyl sulfoxide, and insoluble in other organic solvents. Hydrolysis of the heteropolysaccharides by an endodextranase from a *Penicillium* species was examined in an acetate buffer solution (pH 5.3) at 37°C. The maximum degree of hydrolysis based on the D-glucose units, $(D.H.)_D$, decreased significantly with increasing 2,3,4-trideoxy-DL-*glycero*-hexopyranose units. The observed $(D.H.)_D$ values were in agreement with those calculated from the diad fractions of the original TBLG–BrDBO copolymers by a statistical treatment previously proposed for the enzymatic hydrolysis of DL-dextran.

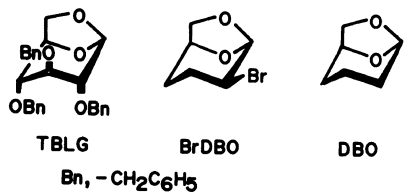
KEY WORDS Synthetic Polysaccharide / Heteropolysaccharide / Ring-Opening Polymerization / Anhydrosugar / Bicyclic Acetal / D-Glucose / Enzymatic Hydrolysis /

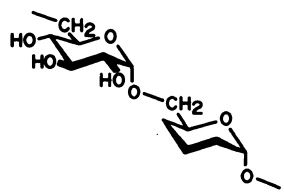
Spectroscopic methods, especially nuclear magnetic resonance spectroscopy, have been frequently used for microstructural analysis of copolymers. In some cases, pyrolysis gaschromatography, oxidative degradation, and selective hydrolysis are effective for that purpose. In addition, enzymatic hydrolysis is often powerful for biological polymers such as polysaccharides and polypeptides. Thus, enzymatic hydrolysis of polysaccharides composed of two or more different structural units occurs selectively at specific glycosidic linkages and therefore provides important information concerning the sequence distributions of the structural units.

Dextranases (α-1,6-glucan-6-glucanohydrolase) cleave (1→6)-α-D-glucosidic linkages of dextran to give isomaltose, (1→6)-α-linked dimer of D-glucose, although some dextranases yield D-glucose as the final product.[1-7] Very recently, we synthesized (1→6)-α-DL-glucopyranan (linear DL-dextran) from noncarbohydrate sources[8] and examined its hydrolysis by an endo-dextranase from a *Penicilium* species.[9] The maximum degree of hydrolysis based on the D-glucose units, $(D.H.)_D$, decreased significantly with decreasing isotactic diad content of the samples, in other words, with decreasing average sequence lengths of the D- and L-glucose units. The observed $(D.H.)_D$ values were reasonably correlated with the tacticities by a simplified

statistical treatment for the enzymatic action on DL-dextran.

As one of a series of works on the chemical synthesis of polysaccharides,[8-18] we describe herein the synthesis and enzymatic hydrolysis of heteropolysaccharides (copolymers) derived from 1,6-anhydro-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranose (TBLG) and *rac*-4(e)-bromo-6,8-dioxabicyclo[3.2.1]octane (BrDBO) by cationic ring-opening copolymerization followed by reductive debromination and subsequent debenzylation. The reductive debromination converts BrDBO units in TBLG–BrDBO copolymers to unsubstituted units identical to those derived by ring-opening polymerization of *rac*-6,8-dioxabicyclo[3.2.1]-octane (DBO). According to the nomenclature of carbohydrate chemistry, DBO is named 1,6-anhydro-2,3,4-trideoxy-*β*-DL-*glycero*-hexopyranose. Therefore, the copolymers after debenzylation are heteropolysaccharides consisting of hydrophilic D-glucose and hydrophobic D- and L-enantiomeric 2,3,4-trideoxy-*glycero*-hexopyranose units, although the structure of the latter unit shown below is of the D-configuration only.




## EXPERIMENTAL

### *Materials*

TBLG was prepared by the pyrolysis of microcrystalline cellulose under reduced pressure[19] followed by the benzylation of the resulting levoglucosan (1,6-anhydro-*β*-D-glucose) in a conventional manner using sodium hydride and benzyl chloride in dimethylformamide. Crude TBLG was purified by recrystallization three times from ethanol and finally from a mixed solvent of *n*-hexane and dichloromethane (2 : 1, v/v): mp 90.5—92.0°C; $[\alpha]_D^{25}$ −31.0° (chloroform) (lit.[20] mp 90—91.5°C; $[\alpha]_D^{25}$ −31.6° (chloroform)). BrDBO was prepared from 3,4-dihydro-2*H*-pyran-2-carbaldehyde (acrolein dimer) by sodium borohydride reduction followed by bromination according to the procedures described by Brown *et al.*[21] with slight modification. The isolation of the 4(e)-isomer and its purification were described in detail in a previous paper.[22]

### *Copolymerization*

Copolymerization of TBLG with BrDBO was carried out in dichloromethane at −60°C with antimony pentafluoride as the initiator. After the reaction was terminated by the addition of a small amount of pyridine, the reaction mixture was poured into a large volume of methanol to precipitate polymer. It was purified by repeated reprecipitation using dichloromethane and methanol as a solvent-precipitant pair, and freeze-dried from a benzene solution. Copolymer compositions were determined by $^1$H NMR and elemental analysis.

### *Reductive Debromination of TBLG-BrDBO Copolymer*

A sample of TBLG–BrDBO copolymer (1.05 g, BrDBO unit 2.4 mmol), azobis(isobutyronitrile) (54 mg, 0.33 mmol) and dry tetrahydrofuran (70 ml) were charged in a three-necked flask equipped with a condenser, a dropping funnel and a nitrogen gas inlet. Under a stream of nitrogen, tri-*n*-butylstannane[23] (5.07 g, 17.4 mmol) was added dropwise with stirring, and the reaction mixture was refluxed for 22 hours. It was concentrated by removing tetrahydrofuran by rotary evap-

oration. The residue was washed repeatedly with *n*-hexane to remove unreacted tri-*n*-butylstannane and tri-*n*-butylbromostannane formed in the reaction. The product was dissolved in a small amount of chloroform and precipitated by the addition of methanol. Subsequent freeze-drying from a benzene solution gave a white powdery polymer. Yield, 0.73 g (93%).

*Debenzylation of TBLG–DBO Copolymer*

A three-necked flask equipped with a cold finger trap and a dropping funnel was immersed in a dry ice–methanol bath. Liquid ammonia (70 ml) was charged in the flask. A sample of reductively debrominated copolymer (1.30 g, TBLG unit 2.3 mmol) dissolved in a mixed solvent of toluene (30 ml) and dimethoxyethane (10 ml) was added through the dropping funnel. The dry ice–methanol bath was removed, and small pieces of sodium metal (1.02 g, 44 mmol) were added in portions to the solution until a dark blue color persisted. After stirring the reaction mixture for 1 hour, a small amount of ammonium chloride was cautiously added to the mixture to decompose the excess sodium, followed by the dropwise addition of water. The finger trap was removed, and stirring was continued until most of the ammonia evaporated. The aqueous and organic layers were separated, and the organic layer was washed several times with water. The water extract combined with the aqueous layer was then extracted with dichloromethane. The aqueous solution was dialyzed in a stream of water for 2 days. It was concentrated by a rotary evaporator and subsequently freeze-dried to yield a white powdery polymer. Yield, 0.49 g (90%).

*Enzymatic Hydrolysis of Heteropolysaccharide*

Enzymatic hydrolysis of heteropolysaccharide was monitored by colorimetric determination of the reducing units of the saccharides produced during hydrolysis by means of Somogy–Nelson's method[24] in a manner similar to the enzymatic hydrolysis of DL-dextran.[9]

*Characterization*

$^1$H and $^{13}$C NMR spectra were recorded on a JEOL FX-200 spectrometer operating at 200 MHz ($^1$H) and 50 MHz ($^{13}$C), respectively, on solutions in deuteriochloroform and deuterium oxide. Tetramethylsilane and acetone were used as the internal references for solutions in deuteriochloroform and deuterium oxide, respectively. Specific rotations were measured in chloroform (TBLG monomer, TBLG–BrDBO and TBLG–DBO copolymers) and in water (heteropolysaccharide) at 25°C by a JASCO DIP 181 automatic polarimeter. Molecular weights of copolymers were estimated by gel permeation chromatography. For TBLG–BrDBO and TBLG–DBO copolymers: column, Shodex A80M (1 m); eluent, chloroform; polystyrene standard. For heteropolysaccharide: column, Shodex OH pak B804 (50 cm) and B805 (50 cm); eluent water; pullulan standard.

## RESULTS AND DISCUSSION

*Synthesis of Heteropolysaccharide*

Copolymerization of TBLG with BrDBO was carried out mainly in dichloromethane at −60°C with antimony pentafluoride as the initiator. Table I summarizes some of the results of the copolymerization. The polymers obtained by the repeated reprecipitation from a dichloromethane solution into methanol were soluble in benzene and chloroform, in contrast to the insolubility of the homopolymer of BrDBO in the former solvent and the poor solubility in the latter solvent. This is clearly indicative of the formation of a true copolymer.

Additional support for the formation of random copolymers was provided by the $^{13}$C NMR spectrum shown in Figure 1. Some of the signals split into two peaks due to the presence of the different diad placements of

**Table I.** Copolymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBLG) with 4(e)-bromo-6,8-dioxabicyclo[3.2.1]octane (BrDBO)[a]

| Expl. No. | TBLG | BrDBO | $F_T$[b] | Solv. | Time | Yield | $\bar{M}_n$[c] × $10^{-4}$ | $\bar{M}_w/\bar{M}_n$[c] | $[\alpha]_D^{25}$[d] | $f_T$[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| | g | g | | ml | min | % | | | | |
| T-17 | 4.32 | 0 | 1.00 | 8 | 90 | 52 | 4.8 | 3.1 | +110 | 1.00 |
| T-36 | 5.84 | 0.29 | 0.90 | 10 | 15 | 28 | 2.0 | 2.0 | +103 | 0.92 |
| OT-2[f] | 1.38 | 0.16 | 0.80 | 3.2 | 5 | 60 | 3.7 | 2.5 | +92 | 0.87 |
| T-37 | 5.18 | 0.58 | 0.80 | 10 | 10 | 27 | 4.0 | 2.5 | +99 | 0.83 |
| T-71[g] | 8.64 | 3.84 | 0.50 | 40 | 60 | 81 | 2.4 | 2.0 | +82 | 0.60 |
| T-63[h] | 2.17 | 0.96 | 0.50 | 10 | 60 | 83 | 3.1 | 1.9 | +75 | 0.52 |
| T-49 | 1.31 | 1.36 | 0.30 | 6 | 45 | 75 | 1.7 | 4.4 | +50 | 0.36 |

[a] Initiator, $SbF_5$, 5 mol% to total monomers; solvent, $CH_2Cl_2$; temperature, −60°C.
[b] Mole fraction of TBLG in monomer feed.
[c] By gel permeation chromatography (polystyrene standard).
[d] In chloroform.
[e] Mole fraction of TBLG unit in copolymer determined by $^1H$ NMR spectroscopy.
[f] Optically active L-BrDBO was used.
[g] Initiator, $PF_5$, 1 mol% to total monomers.
[h] Initiator, $SbF_5$, 3 mol% to total monomers; solvent, 1-nitropropane.

**Table II.** Composition, diad fractions, and sequence lengths of TBLG (T)–BrDBO (B) copolymers

| Copolymer No. | Composition[a] | | Diad fraction[b] | | | $R$[d] | $l_T$[e] | $l_B$[e] |
|---|---|---|---|---|---|---|---|---|
| | $f_T$ | $f_B$ | $f_{TT}$ | $f_{TB}$ | $f_{BB}$ | | | |
| T-17 | 1.00 | 0 | 1.00 | 0 | 0 | — | — | — |
| T-36 | 0.92 | 0.08 | 0.85 | 0.07 | 0.01 | 14 | 13.1 | 1.1 |
| OT-2 | 0.87 | 0.13 | 0.80 | 0.07 | 0.06 | 14 | 12.4 | 1.9 |
| T-37 | 0.83 | 0.17 | 0.71 | 0.12 | 0.05 | 24 | 6.9 | 1.4 |
| T-71 | 0.60 | 0.40 | 0.36 | 0.24 | 0.16 | 48 | 2.5 | 1.7 |
| T-63 | 0.52 | 0.48 | 0.28 | 0.24 | 0.24 | 48 | 2.2 | 2.0 |
| T-49 | 0.36 | 0.64 | 0.15 | 0.21 | 0.43 | 42 | 1.7 | 3.0 |

[a] Determined by $^1H$ NMR spectroscopy.
[b] Determined by $^{13}C$ NMR spectroscopy. See the text.
[c] Calculated as $f_B - f_{TB}$ by assuming $f_{BT} = f_{TB}$.
[d] Run number defined as number of sequences per 100 monomeric units.
[e] Number average sequence length.

TBLG and BrDBO units, although the splittings of the signals ascribable to BrDBO units may be caused also by the different diad placements of the enantiomeric BrDBO units.[25,26] Particularly, the signal j assignable to the benzyl methylene carbon on O(2) of TBLG unit appears as a pair of peaks with different intensities. The higher field signal at δ 72.0 ppm is due to TBLG–TBLG consecutive diad and the lower field signal at δ 72.6 ppm to TBLG–BrDBO crossover diad. Therefore, information on the microstructure of TBLG–BrDBO copolymer can be obtained from the relative areas of these peaks. In Table II are collected diad fractions, run number, and average sequence lengths for the TBLG–BrDBO copolymers.

As a preliminary experiment, a TBLG–BrDBO copolymer was treated with metallic sodium in liquid ammonia, in expectation that

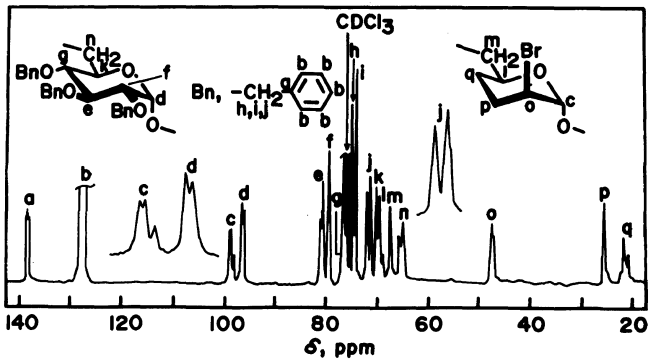
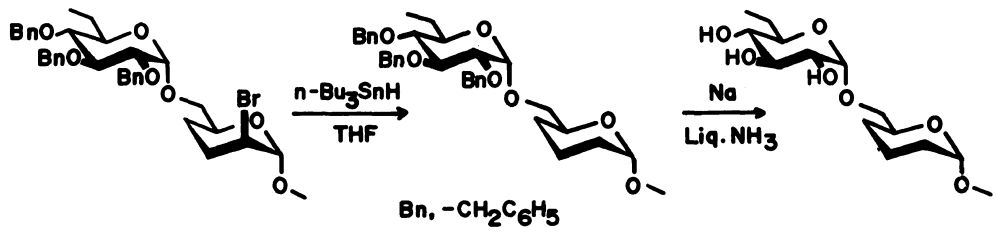



**Figure 1.** $^{13}C$ NMR spectrum of TBLG–BrDBO copolymer prepared in dichloromethane at −60°C with phosphorus pentafluoride as the initiator. Solvent, $CDCl_3$; temp, 50°C; 50 MHz; internal reference, tetramethylsilane.

both debromination and debenzylation could be achieved in one step. However, elimination of hydrogen bromide from BrDBO units occurred concurrently with reductive debromination, resulting in a copolymer containing some dihydropyran rings. Therefore, reductive debromination of BrDBO units and debenzylation of TBLG units were undertaken separately: First, BrDBO units in TBLG–BrDBO copolymer were reductively debrominated with tri-*n*-butylstannane in tetrahydrofuran at reflux temperature. Subsequently, TBLG units in the resulting copolymer were debenzylated by the conventional method using metallic sodium in liquid ammonia.




Tri-*n*-butylstannane has been used for the reductive dechlorination of poly(vinyl chloride).[27-30] As reported previously,[26] the reductive debromination of the homopolymer of BrDBO was successfully performed with this reagent and the tacticity of the resulting polymer identical to the homopolymer of DBO was evaluated by $^{13}C$ NMR spectroscopy. Table III presents the results of the reductive debromination of TBLG–BrDBO copolymers. In most cases, the reductively debrominated copolymers (hereafter referred to as TBLG–DBO copolymers) were obtained in nearly quantitative yields. The number average molecular weights and polydispersity of the copolymers were close to those expected from the original copolymers.

Figure 2 shows a typical $^{13}C$ NMR spectrum of TBLG–DBO copolymer. The signals were assigned on the basis of the reported chemical shifts for the homopolymers of TBLG and DBO. Neither the signal ($\delta$ 48.1 ppm) assignable to the methine carbon bearing a bromine atom in the original copolymer nor olefinic carbon signals appear in the spectrum, indicating that the reductive debromination pro-

**Table III.** Reductive debromination of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBLG)–4(e)-bromo-6,8-dioxabicyclo[3.2.1]octane (BrDBO) copolymer[a]

| Copolymer[b] | | Solv. | $n$-$Bu_3SnH$ | AIBN[c] | Time | Yield | $\bar{M}_n \times 10^{-4}$ [d] | $\bar{M}_w/\bar{M}_n$ [d] | $[\alpha]^{25}$ [e] | $f_T$ [f] |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | g | ml | g | mg | h | % | | | | |
| T-36 | 1.47 | 80 | 0.90 | 14 | 24 | 100 | 1.5 | 1.9 | +102 | 0.91 |
| OT-2 | 0.48 | 30 | 0.32 | 12 | 24 | 100 | 2.5 | 2.2 | +96 | 0.87 |
| T-37 | 1.06 | 70 | 2.38 | 54 | 24 | 93 | 3.1 | 2.3 | +98 | 0.83 |
| T-71 | 3.01 | 100 | 3.11 | 37 | 20 | 85 | 1.8 | 2.1 | +75 | 0.61 |
| T-63 | 2.42 | 100 | 5.00 | 36 | 24 | 99 | 2.0 | 1.8 | +64 | 0.52 |
| T-49 | 1.05 | 70 | 5.07 | 54 | 22 | 93 | 1.5 | 4.6 | +47 | 0.34 |

[a] Solvent, tetrahydrofuran; at reflux temperature.
[b] Sample numbers correspond to the experimental numbers in Table I.
[c] Azobis(isobutyronitrile).
[d] By gel permeation chromatography (polystyrene standard).
[e] In chloroform.
[f] Mole fraction of TBLG unit in copolymer determined by $^1H$ NMR spectroscopy.

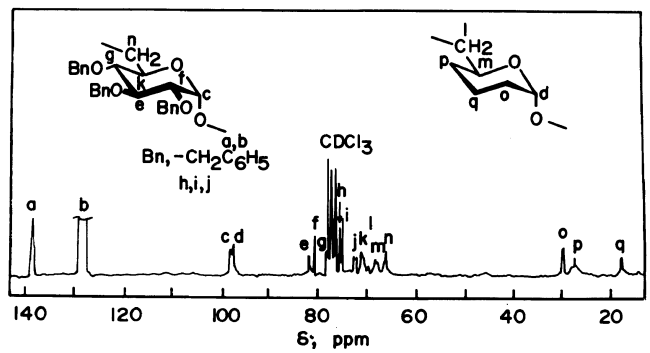



**Figure 2.** $^{13}C$ NMR spectrum of TBLG–DBO copolymer obtained by reductive debromination of TBLG–BrDBO copolymer. Solvent, $CDCl_3$; temp, 50°C; 50 MHz; internal reference tetramethylsilane.

ceeded to completion without accompanying side reactions. The appearance of the split signals e, j, and m is ascribable to the presence of different diad placements as observed in the original TBLG–BrDBO copolymer. However, the resolution of each of these pairs of peaks was too small to allow reliable determination of diad fractions. The TBLG–DBO copolymer thus obtained was readily soluble in benzene and chloroform. It is to be noted here that TBLG–DBO random copolymer cannot be prepared directly by cationic ring-opening copolymerization of TBLG with DBO, because the latter monomer is much more reactive than the former.

As the second step, TBLG–DBO copolymers of various compositions were debenzylated by metallic sodium in liquid ammonia. The results of the debenzylation were listed in Table IV. Even the copolymer with a mole fraction of D-glucose unit of 0.34 was soluble in water and dimethyl sulfoxide, and insoluble in benzene and chloroform.

Figure 3 displays the $^{13}C$ NMR spectrum of the debenzylated copolymer. No aromatic carbon signals appear in the spectrum, indicating that the debenzylation was perfectly achieved. The signals were assigned as shown in the figure on the basis of the assignments of the $^{13}C$ NMR signals for dextran and the homo-

**Table IV.** Debenzylation of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (TBLG)-6,8-dioxabicyclo[3.2.1]octane (DBO) copolymer

| Copolymer[a] | | $NH_3$ | Totuene | DME[b] | Na | Yield | $\bar{M}_n \times 10^{-3}$ [c] | $\bar{M}_w/\bar{M}_n$ [c] | $[\alpha]_D^{25}$ [d] deg | $f_G$ [e] |
|---|---|---|---|---|---|---|---|---|---|---|
| No. | g | ml | ml | ml | g | % | | | | |
| T-17 | 1.50 | 150 | 45 | 15 | 1.01 | 93 | 12.3 | 1.6 | +180 | 1.00 |
| T-36 | 1.30 | 100 | 30 | 10 | 1.02 | 90 | 7.3 | 2.0 | +158 | 0.90 |
| OT-2 | 0.40 | 50 | 15 | 5 | 0.30 | 85 | 12.0 | 1.9 | +140 | 0.89 |
| T-37 | 1.68 | 150 | 45 | 15 | 0.81 | 90 | 11.2 | 2.0 | +145 | 0.83 |
| T-71 | 1.58 | 150 | 45 | 15 | 0.75 | 65 | 12.7 | 2.0 | +126 | 0.61 |
| T-63 | 1.59 | 150 | 45 | 15 | 0.50 | 61 | 10.1 | 2.1 | +97 | 0.52 |
| T-49 | 0.38 | 50 | 15 | 7 | 0.40 | 60 | 6.9 | 1.9 | — | 0.34 |

[a] Sample numbers correspond to those in Table II.
[b] 1,2-Dimethoxyethane.
[c] By gel permeation chromatography (pullulan standard).
[d] In water.
[e] Mole fraction of glucose unit in copolymer determined by $^1H$ NMR spectroscopy.

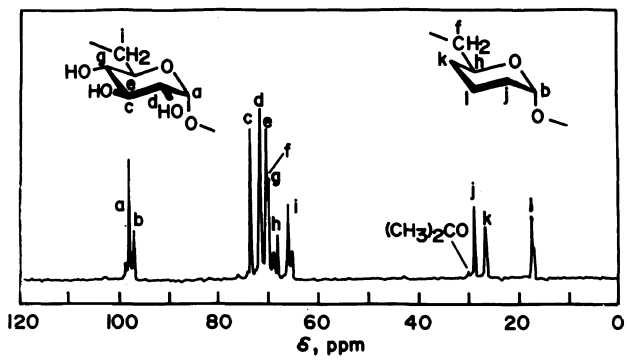



**Figure 3.** $^{13}C$ NMR spectrum of (1→6)-α-linked heteropolysaccharide consisting of D-glucose and 2,3,4-trideoxy-DL-*glycero*-hexopyranose units prepared by debenzylation of TBLG–DBO copolymer. Solvent, $D_2O$; temp, 70°C; 50 MHz; internal reference, acetone.

polymer of DBO. Since the DBO unit in the copolymer is named 2,3,4-trideoxy-(1→6)-α-DL-*glycero*-hexopyranosyl residue according to the nomenclature of carbohydrate chemistry, the copolymer is hereafter referred to as heteropolysaccharide.

Strictly speaking, the D- and L-enantiomer contents of the 2,3,4-trideoxy-*glycero*-hexopyranose units in the heteropolysaccharide are not equal. In the copolymerization of TBLG with racemic BrDBO, asymmetric selection of BrDBO monomer takes place to some extent:[22] Thus, the D-enantiomer of BrDBO is more favorably incorporated into the copolymer. Therefore, the heteropolysaccharide derived from TBLG–BrDBO copolymer is somewhat rich in the D-enantiomer of 2,3,4-trideoxy-*glycero*-hexopyranose.

*Enzymatic Hydrolysis*

Very recently, we have synthesized DL-dextran having different tacticities and examined their hydrolysis by an endo-dextranase.[9] The maximum degree of hydrolysis based on the D-glucose units, $(D.H.)_D$, decreased significantly with decreasing isotactic diad content

of the DL-dextrans. The experimental results were reasonably interpreted by a statistical treatment of the enzymatic action on the DL-dextrans.

The heteropolysaccharides described in the foregoing section consist of (1→6)-α-D-glucopyranosyl and 2,3,4-trideoxy-(1→6)-α-DL-*glycero*-hexopyranosyl residues, the latter of which should affect the enzymatic hydrolysis of the former units depending on the microstructures of the heteropolysaccharides. The enzymatic hydrolysis of the heteropolysaccharides was carried out in an acetate buffer solution (pH 5.3) at 37°C. An endo-dextranase from a *Penicillium* species was used as the enzyme.

Figure 4 represents graphically the enzymatic hydrolysis of the heteropolysaccharides of three different compositions, along with synthetic linear dextran for comparison. The linear dextran was derived from the homopolymer of TBLG (T-17). In each run, the degree of hydrolysis represented as the percentage of the hydrolyzed glucosidic linkages to the number of D-glucose units in the sample reached a constant value after 3 days. In some experiments, it was confirmed that the addition of a fresh solution of the enzyme to a reaction mixture at this stage did not increase the degree of hydrolysis. Therefore, the degree of hydrolysis attained after 3 days can be regarded as the maximum degree of hydrolysis based on the D-glucose unit, $(D.H.)_D$.

Dextranases from different sources exhibit diverse action patterns, but fundamentally they hydrolyze natural dextran to isomaltose, (1→6)-α-linked dimer of D-glucose.[1-7] Taking this fact into consideration and making several bold assumptions, we derived eq 1 expressing the $(D.H.)_D$ values for DL-dextrans.

$$(D.H.)_D = P_{DL}\sum \bar{N}_n'' P(D)_n \times 100 \qquad (1)$$

where $P_{DL}$ is the conditional probability for a D-glucose unit, D, to be followed by a L-glucose unit, L. $\bar{N}_n''$ is the expected number of bond

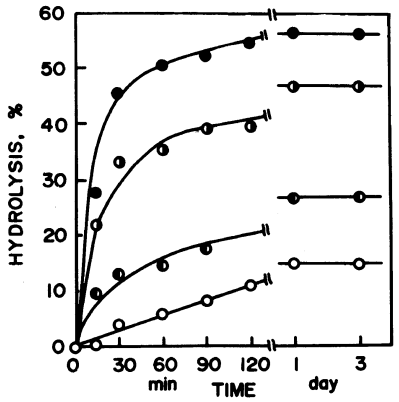



**Figure 4.** Hydrolysis of (1→6)-α-linked heteropolysaccharides composed of D-glucose and 2,3,4-trideoxy-DL-*glycero*-hexopyranose units by endo-dextranase. Hydrolysis % is defined as the percentage of the hydrolyzed glucosidic linkages to the D-glucose units in the samples. Acetate buffer solution, pH 5.3; temp, 37°C; [substrate], $(7.4—9.0)\times 10^2\,mg\,l^{-1}$; [dextranase], $0.4\,mg\,l^{-1}$. Mole fraction of D-glucose unit: ●, 1.0; ◑, 0.83; ◐, 0.61; ○, 0.52.

cleavages for the sequence of $LD_nL$, and $P(D)_n$ is the fraction of the $D_n$ sequence defined by eq 2.

$$P(D)_n = P_{DD}^{n-1} P_{DL} \qquad (2)$$

where $P_{DD}$ is the conditional probability for a D-glucose unit to be followed by a D-glucose unit ($P_{DD} = 1 - P_{DL}$).

In order to apply eq 1 to the enzymatic hydrolysis of the heteropolysaccharides, we need the corresponding conditional probabilities for each sample. Strictly speaking, the heteropolysaccharides are terpolymers consisting of D-glucose and D- and L-enantiomeric 2,3,4-trideoxy-*glycero*-hexopyranose units. If the enzyme interacts with the latter two units in different manners, these D- and L-enantiomeric units must be treated as different units. However, the $(D.H.)_D$ values of the heteropolysaccharide (OT-2) composed of D-glucose and 2,3,4-trideoxy-L-*glycero*-hexopyranose units was close to the $(D.H.)_D$ value for the heteropolysaccharide (T-36) with a similar mole fraction of D-glucose unit and containing both D- and L-enantiomeric 2,3,4-trideoxy-*glycero*-hexopyranose units. This

**Table V.** Enzymatic hydrolysis of heteropolysaccharides consisting of D-glucose (D) and 2,3,4-trideoxy-DL-*glycero*-hexopyranose (G) by endo-dextranase[a]

| Sample No. | [Substrate] $\times 10^{-2}$/mg l$^{-1}$ | $f_D$[b] | $P_{DD}$[c] | $P_{DG}$[c] | $(D.H.)_D$[d] | |
|---|---|---|---|---|---|---|
| | | | | | Found | Calcd |
| Clinic[e] | 5.8 | (1.00) | (1.00) | (0) | 52.2 | 56.5[f] |
| T-17 | 7.4 | (1.00) | (1.00) | (0) | 56.0 | 56.5[f] |
| T-36 | 8.0 | 0.90 | 0.92 | 0.08 | 50.7 | 50.4 |
| OT-2[g] | 3.2 | 0.89 | 0.92 | 0.08 | 50.4 | 50.0 |
| T-37 | 8.0 | 0.83 | 0.86 | 0.14 | 47.5 | 45.5 |
| T-71 | 8.6 | 0.61 | 0.60 | 0.40 | 28.4 | 26.2 |
| T-63 | 9.0 | 0.52 | 0.54 | 0.46 | 21.5 | 22.0 |
| T-49 | 8.6 | 0.34 | 0.42 | 0.58 | 14.7 | 14.2 |

[a] Temperature, 37°C; pH, 5.3; 0.1 M acetate buffer; [dextranase], 0.4 mg l$^{-1}$.
[b] Mole fraction of D-glucose unit in the heteropolysaccharide determined by $^1$H NMR spectroscopy.
[c] Conditional probabilities for a D-glucose unit to be followed by a D-glucose unit and by a 2,3,4-trideoxy-*glycero*-hexopyranose unit, respectively. See the text.
[d] Maximum degree of hydrolysis based on the D-glucose units in the heteropolysaccharides.
[e] $\bar{M}_n = 16{,}000$. A commercial clinical dextran.
[f] Calculated as $\bar{N}_n/n$ ($n = 100$), where $\bar{N}_n$ is the expected average number of bond cleavages for a hypothetical linear dextran with the degree of polymerization of $n$.[9]
[g] Heteropolysaccharide composed of D-glucose and 2,3,4-trideoxy-L-*glycero*-hexopyranose units.

means that the enzyme does not differentiate these D- and L-enantiomeric trideoxy units. Therefore, the heteropolysaccharides were treated as binary copolymers for the sake of simplification.

Since information on the microstructures of the heteropolysaccharides cannot be obtained directly from their $^{13}$C NMR spectra, the conditional probabilities $P_{DG}$ and $P_{DD}$ needed for the calculation of the $(D.H.)_D$ values were determined from the microstructural data for the corresponding TBLG-BrDBO copolymers given in Table II, assuming that neither the reductive debromination nor the debenzylation of the copolymers altered their microstructures.

$$P_{DG} = f_{TB}/f_T \quad (3)$$

$$P_{DD} = 1 - P_{DG} \quad (4)$$

where $P_{DG}$ and $P_{DD}$ are the conditional probabilities for a D-glucose unit to be followed by a 2,3,4-trideoxy-*glycero*-hexopyranose unit, G, and by a D-glucose unit, respectively, in the heteropolysaccharide. $f_{TB}$ and $f_T$ are the mole fractions of TBLG–BrDBO diad and TBLG unit, respectively, in the TBLG–BrDBO copolymer.

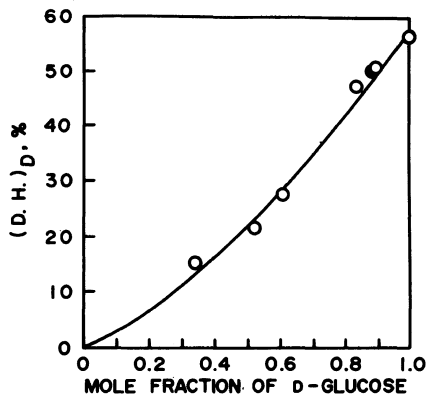



**Figure 5.** Composition dependence of maximum degree of hydrolysis based on D-glucose units, $(D.H.)_D$, in the enzymatic hydrolysis of (1→6)-α-linked heteropolysaccharides composed of D-glucose and 2,3,4-trideoxy-*glycero*-hexopyranose units. Acetate buffer solution, pH 5.3; temp, 37°C; [substrate], (3.2—9.0) $\times 10^2$ mg l$^{-1}$; [dextranase], 0.4 mg l$^{-1}$; ○, DL-*glycero*; ●, L-*glycero*; ——, calculated curve.

Using the $P_{DG}$ and $P_{DD}$ values thus obtained and the numerical values of $\bar{N}_n''$ given in Table I of the previous paper,[9] the $(D.H.)_D$ value for each sample was estimated by eq 1. The observed and calculated values are compared in Table V. The data for clinical and synthetic linear dextrans are also included in Table V.

The agreement between the observed and calculated $(D.H.)_D$ values are as a whole satisfactory, considering the approximations involved in the calculation. The somewhat large discrepancy for the clinical dextran undoubtedly arises from its branched structures,[31,32] since the agreement for the synthetic linear dextran is excellent.

Figure 5 shows the dependence of the $(D.H.)_D$ value on the mole fraction of D-glucose unit in the heteropolysaccharides. Clearly, the $(D.H.)_D$ value decreases rapidly with increasing 2,3,4-trideoxy-*glycero*-hexopyranose unit which does not interact with the endo-dextranase.

In the derivation of eq 1, the following assumptions were made.[9] 1) Among the four D–D, D–L, L–D, and L–L glucosidic linkages, only the D–D linkage is susceptible to enzymatic hydrolysis. 2) The D–D glucosidic linkage inserted between L-glucose units in a $LD_2L$ sequence is not hydrolyzed by the dextranase. The good agreement between the observed and calculated $(D.H.)_D$ values as demonstrated in Table V and Figure 5 implies that similar assumptions are valid also in the enzymatic hydrolysis of the (1→6)-α-linked heteropolysaccharides composed of D-glucose and 2,3,4-trideoxy-DL-*glycero*-hexopyranose units: Only the glucosidic linkages between two consecutive D-glucose units are cleaved, except those inserted between two 2,3,4-trideoxy-*glycero*-hexopyranose units in a $GD_2G$ tetrad sequence. Accordingly, the binding of 2,3,4-trideoxy-*glycero*-hexopyranose units to the vicinity of the active site of the enzyme hardly occur or does not occur at all. The significant depression of the $(D.H.)_D$ values with decreasing D-glucose content in the heteropolysaccharides unequivocally indicates that the average sequence length of D-glucose units becomes rapidly short as the 2,3,4-trideoxy-*glycero*-hexopyranose content increases. This is clearly observable in the $\bar{l}_T$ values for the TBLG–BrDBO copolymers in Table II. The foregoing results demonstrate that the enzymatic hydrolysis is informative on the microstructures of the heteropolysaccharides.

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