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Speciality Polymers Having Sugar as the Pendant Group: Synthesis, Characterization, and Binding of Organic Solute in Water

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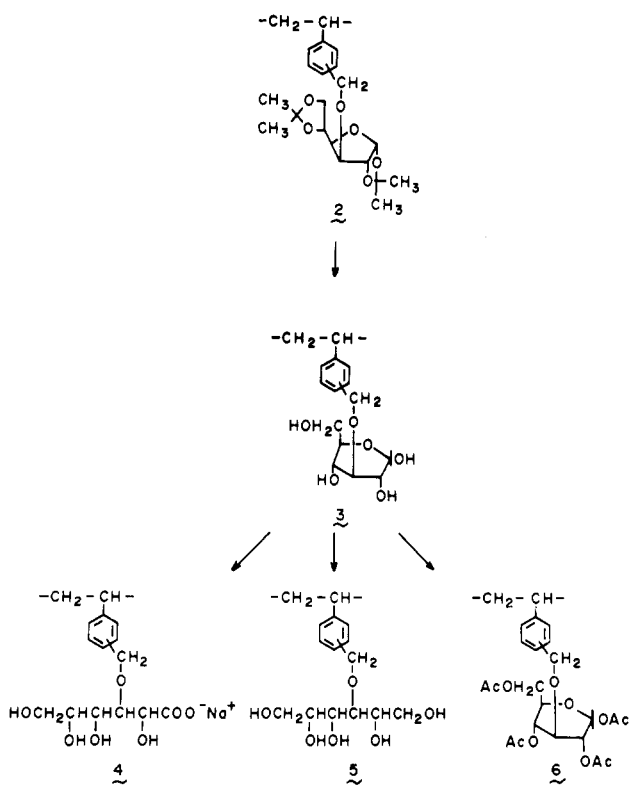
ABSTRACT: A high molecular weight polymer **3** consisting of a sugar and styrene derivative was prepared by radical polymerization of the hydroxyl-blocked monomer **1** followed by deblocking of the resulting polymer **2**. Oxidation, reduction, and acetylation of the sugar moiety of **3** were performed, and the polymers obtained were characterized. The amphiphilic polymer **3** was found to act as a neutral polysoap in water. The interaction between **3** and methyl orange was spectrophotometrically investigated, leading to a finding that methyl orange was strongly bound to the hydrophobic region of **3** in water. Binding constants were determined according to the procedures suggested by Benesi-Hildebrand and Klotz to compare with those of other polymers. It was suggested that intramolecular aggregation of the vinylbenzyl residues occurred to form hydrophobic regions which were enclosed in hydrophilic surroundings of sugar residues.

Much attention has been paid to polymers derived from vinyl derivatives of sugars.[1-4] Since polypeptide synthesis on polymer supports was reported by Merrifield,[5] sugar polymers have been used as intermediates in the solid-phase synthesis of carbohydrates,[6-9] in particular of oligosaccharides.[10-20] Besides sugar synthesis, recent attention is being focused on the preparation and use of speciality polymers which are endowed with specific functions of sugars. Chiral templates derived from sugars were useful for asymmetric synthesis and optical resolution of organic molecules.[21-24] The hydrophilicity of sugar was applied to the design of a reverse osmosis membrane[25] and a selectively-permeable membrane.[26] Linear, water-soluble sugar polymers are also attractive for potential elucidation of biological roles of carbohydrates and their pharmacological and physiological applications.[27,28] Other attempts have also been made to develop various kinds of sugar polymers.[29-33]

The present investigation has been undertaken to develop a new type of speciality polymer consisting of sugar and styrene derivatives as shown in Scheme I, with the expectation that they might possess novel properties differing from those of naturally occurring polysaccharides and synthetic polymers. The oxygen in position 3 of sugar derivatives is covalently attached to the main chain through a benzyl ether linkage which is chemically stable even in acid and alkaline media. The hydroxyl-blocked polymer **2**, prepared by radical polymerization of the corresponding monomer **1**, was converted to the polymer **3** by removal of the blocking group. The polymer **3**, having a reducing sugar in its repeating unit, is subject to various chemical modifications. Oxidation, reduction, and acetylation of **3** resulted in the polymers **4**, **5**, and **6**, respectively, in excellent yields. It is worth noting that the repeating structural unit of **3**, as well as **4** and **5**, is composed of a hydrophobic vinylbenzyl residue and a hydrophilic sugar derivative. These amphiphilic polymers are expected to show some specific behaviors in water. It is suggested that the organic solute methyl orange was bound to the hydrophobic regions of **3** in water. The binding properties are discussed and compared with those of other polymers.[34-40]

Scheme I
Reaction Scheme of Speciality Polymers Having Sugar Derivatives as the Pendant Group



Experimental Section

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose-3-oxymethylstyrene (1). 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (diacetoneglucose)[41] (mp 109-110 °C) (26 g, 0.1 mol) was dissolved in 150 mL of dry dimethylformamide (DMF) in a 500-mL three-necked round-bottomed flask and was treated with 5.1 g (0.11 mol) of sodium hydride oil suspension at room temperature for 2 h. Chloromethylstyrene (30:70 para and meta isomeric mixture commercially available from Tokyo Chemical Industry Co., Ltd.) (15.2 g, 0.1 mol) was added gradually. The reaction mixture was heated in an oil bath at 50 °C for 6 h. The brown solution was transferred into a separatory funnel together with 300 mL of benzene and was extracted with four 100-mL portions of water. The benzene solution was concentrated in a rotary evaporator and steam distilled to remove unreacted chloromethylstyrene. The residue was dissolved in 30 mL of chloroform and washed with 200 mL of water until little diacetoneglucose was detected by TLC. The residue was diluted with chloroform, treated with charcoal, and dried under vacuum. The yield was 26-28 g (70-75%).

The crude product was chromatographed over silica gel with benzene-*n*-hexane (5/1 v/v) and then benzene-ether (5/1 v/v)

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Table I
Preparation of 2[a]

expt	monomer, g	AIBN, mol %	time, h	yield, g	yield, %	$[\eta]^b$
M1	3.76	0.50	9	2.07	55.1	0.68
I8	3.78	0.26	13	1.71	45.5	0.89
K24	3.76	0.10	17	1.16	30.9	0.93

[a] Solvent, benzene, 5 mL; temperature, 60 °C. [b] Determined in benzene at 25 °C.

as solvents. The eluate was monitored by TLC (benzene–ether, 5/1 v/v). The eluate containing pure monomer with an R_f value of 0.58 was collected, concentrated, and dried under reduced pressure: n^{25}_D 1.5109; UV (*n*-hexane) λ_{max} 284 nm (ϵ_{max} 760) and 294 nm (ϵ_{max} 530); ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 4 H, aromatic -CH-), 6.71 (d × d, 1 H, vinyl -CH=, J = 17 and 10.5 Hz), 5.90 (d, 1 H, α-furanose H-1, J = 4.5 Hz), 5.77 (d, 1 H, vinyl CH_2=, J = 17 Hz), 5.27 (d, 1 H, vinyl CH_2=, J = 10.5 Hz), 4.68 (s, 2 H, benzyl -CH_2-), 4.62 (m, 1 H, H-2), 4.38 and 4.13 (m, 5 H), 1.49, 1.43, 1.37, and 1.32 (four singlets, 12 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.86, 137.71, 137.14, 136.59, and 136.39 (aromatic -C= and vinyl -CH=), 128.50, 127.81, 126.99, 126.16, 125.63, and 125.43 (aromatic -CH=), 114.03 (vinyl CH_2=), 111.69 and 108.91 (>C(O)(O)), 105.26 (C-1), 82.60, 81.62, 81.28, 72.46, and 72.12 (C-2–5, benzyl CH_2), 67.35 (C-6), 26.80, 26.22, and 25.44 (CH_3). Anal. Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50; mol wt 376.4. Found: C, 66.49; H, 7.30; mol wt 381 (vapor pressure osmometry in benzene at 37 °C).

Poly(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-3-oxymethylstyrene) (2). Purified monomer **1** (0.01 mol), a given amount of azobis(isobutyronitrile), and benzene (5 mL) were charged in a glass ampule. The mixed solution was frozen in a solid carbon dioxide–methanol bath and degassed three times. The ampule was sealed under reduced pressure and maintained in a thermostat at 60 ± 0.05 °C. The solution was chilled and poured into cold methanol. The polymer was reprecipitated from its benzene solution into methanol three times and freeze-dried from its benzene solution. The polymerization data are summarized in Table I.

The polymer **2** was soluble in chloroform, benzene, acetone, pyridine, DMF, and dimethyl sulfoxide (Me_2SO). The differential thermal analysis (DTA) showed glass transition at about 90 °C and the decomposition point at 160–180 °C: ^{1}H NMR ($CDCl_3$) δ 7.00 and 6.38 (two broad singlets, 4 H, aromatic), 5.87 (broad singlet, 1 H, α-furanose H-1), 4.55, 4.36, 4.20, and 4.04 (four broad singlets, 8 H), 1.52, 1.44, and 1.32 (15 H, CH_3 and -CH_2CH-); ^{13}C NMR ($CDCl_3$) δ 144.63, 137.17, 134.93, and 127.38 (aromatic), 111.69 and 108.86 (>C(O)(O)), 105.21 (α-furanose C-1), 82.45, 82.11, 81.23, and 72.51 (C-2–5, benzyl -CH_2-), 67.30 (C-6), 44.0 and 40.7 (-CH_2CH-), 26.85, 26.31, and 25.58 (CH_3). Anal. Calcd for $(C_{21}H_{28}O_6)_n$: C, 67.00; H, 7.50. Found: C, 67.07; H, 7.51.

Poly(D-glucopyranose-3-oxymethylstyrene) (3). Christensen–Goodman's procedure[42] was modified. The polymer **2** (0.50 g) was dissolved in 5 mL of a trifluoroacetic acid–water (0.85:0.15 v/v) mixture. The solution was stirred magnetically at room temperature for 40 min, diluted with 100 mL of water, dialyzed overnight in a cellulose tube in the dark, and concentrated in a rotary evaporator. After freeze-drying, **3** was isolated as a white powder: yield 0.387 g (98%); soluble in water, Me_2SO, DMF, and pyridine; decomposition point about 200 °C; ^{13}C NMR (Me_2SO-d_6) δ 144.1, 138.9, 136.3, and 126.5 (aromatic), 96.05 (β-pyranose C-1), 92.20 (α-pyranose C-1), 84.64 (β-pyranose C-3), 82.11 (α-pyranose C-3), 76.5, 74.5, 71.9, and 69.7 (C-2, C-4–5, benzyl CH_2), 60.91 (C-6).

Poly(sodium D-gluconate-3-oxymethylstyrene) (4). A solution of **3** (0.47 g) in water (150 mL) was stirred at room temperature and treated alternately and dropwise with aqueous 0.1 N iodine solution and 0.1 N sodium hydroxide solution.[43] In order to follow the progress of the reaction, a 1-mL aliquot was pipetted and acidified with 0.2 N hydrochloric acid, and the residual iodine was checked with 0.1 N sodium thiosulfate solution and starch indicator. The reaction was continued until the presence of an excess amount of iodine was confirmed. Usually, a total of 30 mL of the iodine and 45 mL of the sodium hydroxide was added during 1 h. The solution was dialyzed for 2 days and concentrated in a rotary evaporator under reduced pressure (1 mmHg). The precipitate (0.30 g) was separated by centrifugation and the supernatant was freeze-dried (0.17 g). The total yield was 89%. The product was soluble in water, Me_2SO, and pyridine.

Poly(D-glucitol-3-oxymethylstyrene) (5). Sodium borohydride (2.0 g) was added to a solution of **3** (0.55 g) in water (150 mL). The solution was stirred at room temperature for 2 days and dialyzed in a cellulose tube for 5 days. Insoluble residue was filtered off and the filtrate was concentrated in an evaporator under reduced pressure (1 mmHg). Freeze-drying produced a white powdery polymer **5**: yield 0.53 g (97%); soluble in water, Me_2SO, and pyridine; ^{13}C NMR (Me_2SO-d_6) 143.90, 138.59, 136.10, and 126.75 (aromatic), 78.41 (C-3), 72.71 and 71.19 (C-2, C-4–5, benzyl CH_2), 63.35 and 62.42 (C-1, C-6).

Poly(1,2,4,6-tetra-*O*-acetyl-D-glucopyranose-3-oxymethylstyrene) (6). Freshly prepared **3** (0.64 g) was dissolved into 60 mL of pyridine and treated with 60 mL of acetic anhydride at room temperature for 2 days. The reaction was completed by heating the solution at 80 °C for 5 h. Insoluble residue was removed by filtration through Celite and the filtrate was poured into 600 mL of cold water. The precipitate was collected by centrifugation, washed with water, and dried in vacuo. The crude product was purified by reprecipitation from its chloroform solution into methanol: yield 0.93 g (93%); soluble in chloroform, acetone, Me_2SO, DMF, and pyridine; ^{13}C NMR ($CDCl_3$) 170.46, 169.19, 168.94, and 168.75 (C=O), 144.5, 137.3, 135.0, and 127.3 (aromatic), 91.91 (β-anomer C-1), 89.32 (α-anomer C-1), the region around 80 ppm was concealed behind the solvent peaks, 62.08 (C-6), 41.7, and 40.3 (CH_2CH), 20.71 (CH_3). Anal. Calcd for $(C_{23}H_{28}O_{10})_n$: C, 59.47; H, 6.08. Found: C, 59.23; H, 6.02.

Determination of Acetyl Content. The method of Kunz[44] was modified. The polymer sample (50 mg) was weighed and dissolved in 10 mL of acetone in a 100 mL stop-cocked flask. The solution was cooled in an ice bath, and 10 mL of 0.1 N aqueous sodium hydroxide solution was added drop by drop. After 2.5 h, another 10 mL of 0.1 N sodium hydroxide was added to dissolve a precipitate of the deacetylation product. The mixture was kept at 0 °C for 18 h. A control run was made at the same time. The solutions were titrated with 0.1 N hydrochloric acid; found 8.45 mmol/g; calcd 8.61 mmol/g.

Characterization. NMR spectra were recorded with Japan Electro-Optic Laboratory JNM-MH-100 NMR and JNM-FX-100 Fourier transform NMR spectrometers. Tetramethylsilane was used as an internal standard in deuteriochloroform and Me_2SO-d_6 and as an external standard in deuterium oxide. IR spectra were measured as KBr disks with a JASCO IR-G grating infrared spectrophotometer. Optical rotations were determined in a JASCO DIP-4 automatic polarimeter by using a 1-dm cell. Gel-permeation chromatography (GPC) was carried out by using a Shodex 802A (8 i.d. × 1000 mm) column on a Hitachi 634A high-speed liquid chromatograph (solvent, chloroform). Viscosity was measured in an Ubbelohde viscometer at 25 °C. TLC was carried out on Merck silica gel $60F_{254}$ coated plates and detection was effected by charring with a $CeSO_4$–3.6 N sulfuric acid mixture.

Measurement of Methyl Orange Binding. The binding was measured at room temperature with a JASCO UVIDEC 505 digital double-beam spectrophotometer. Reagent grade methyl orange was used without further purification. The molar absorbance at 464 nm was 25100. The polymer **3** used was derived from the polymer **2** which was obtained under the same conditions as experiment No. M1 in Table I. Methyl orange stock solutions (1×10^{-4} and 0.5×10^{-4} M) in phosphate buffer at pH 6.88 were prepared as reference solutions and as solvents of the polymer sample. Polymer concentrations were expressed on the basis of a repeating structural unit. The difference spectra were measured under the following conditions: scanning rate 12.5 nm/min, slit width 1.0 nm, and extension scale in optical density ×5.

Results and Discussion

Polymerization, Polymer Reaction, and Characterization. Monomer **1** was prepared from diacetoneglucose and commercial chloromethylstyrene (para and meta isomeric mixture). Its polymerization was carried out with the use of azobis(isobutyronitrile) as the initiator in benzene at 60 °C (Table I). The polymerization was

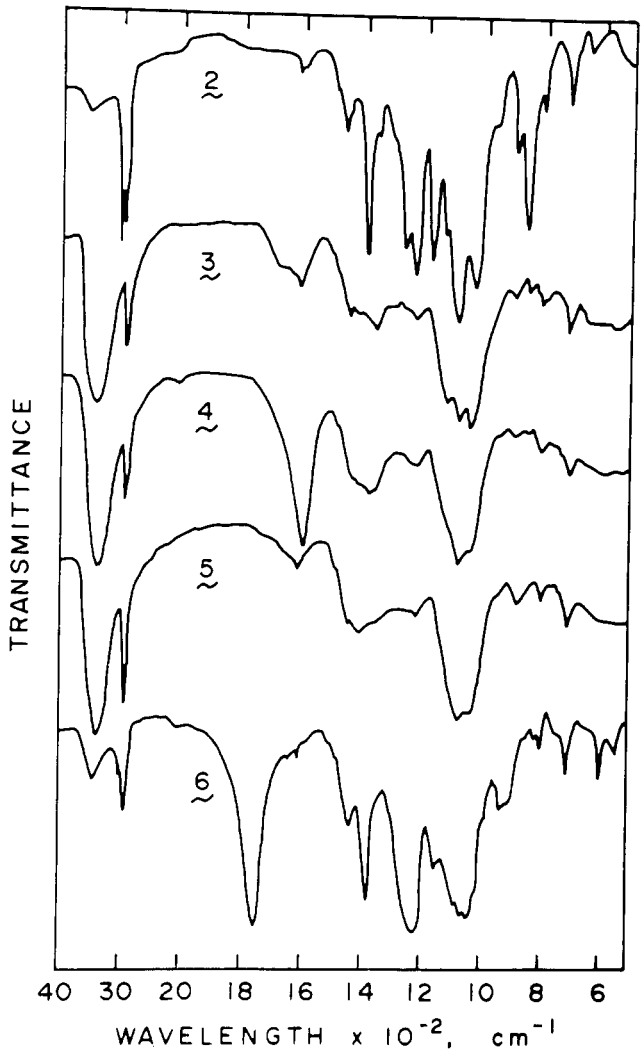

Figure 1. IR spectra of the polymers using a KBr disk. The numbers correspond to those of the polymers in Scheme I.

terminated at a moderate conversion since the product became insoluble in any organic solvents when the conversion increased to higher than 60%. Hydrolysis of the polymer **2** was performed in a mixture of trifluoroacetic acid and water (0.85/0.15 v/v) at room temperature. Stirring the solution for 40 min gave completely hydrolyzed polymer **3**. Gluconate polymer **4** was obtained by oxidation of **3** with sodium hypoiodite, which was generated by mixing 0.1 N iodine solution and 0.1 N sodium hydroxide solution dropwise. Reduction of **3** was carried out in aqueous sodium borohydride solution at room temperature to give **5**. Acetylated polymer **6** was prepared by treating **3** with pyridine and acetic anhydride. All the reactions gave excellent yields of white powdery polymers. It is suggested from the following spectral and analytical data that the reactions attempted went essentially to completion.

The IR spectra of these polymers are presented in Figure 1. The polymer **2** had distinct bands due to the isopropylidene group at 2980, 1385, and 1375 cm^{-1}. In the spectrum of **3**, however, there was no isopropylidene bands and instead there appeared a broad and strong band due to the hydroxyl group at 3400 cm^{-1}. A band at 1600 cm^{-1} of **4** was assignable to the carboxylate group and those at 1750, 1375, and 1220 cm^{-1} of **6** were due to the acetyl group.

Some of the ^1H NMR spectral data are given in the Experimental Section. Signals due to isopropylidene group, appearing in the spectrum of **2**, completely disappeared in the spectrum of **3**. In the spectrum of **6**, there was a large peak around δ 2.0 attributable to four acetyl groups and a methylene and methine backbone in a repeating unit. The area ratio of the peak to others was 1.15, which agreed with the calculated value (15/13) for the completely acetylated polymer. The acetyl content of **6** determined by the method of Kunz[44] also agreed with its theoretical value as described in the Experimental Section. It is clear from these data that the removal of the isopropylidene group and the subsequent acetylation were performed quantitatively.

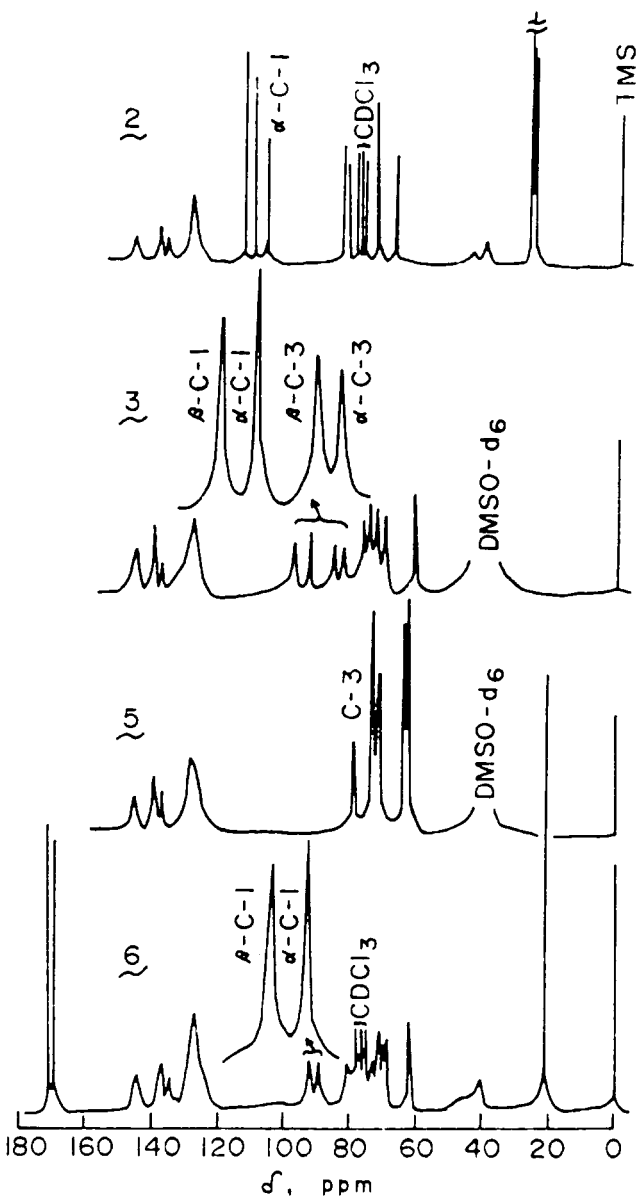

Figure 2. ^{13}C NMR spectra of the polymers: **2** and **6**, in $CDCl_3$: **3** and **5**, in Me_2SO-d_6. The numbers correspond to those of the polymers in Scheme I.

The ^{13}C NMR spectra of **2**, **3**, **5**, and **6** are compared in Figure 2, although **4** could not be measured owing to its insufficient solubility. Deuteriochloroform was used as the solvent for **2** and **6**, and Me_2SO-d_6 was used for **3** and **5**. The chemical shifts and their assignments are presented in the Experimental Section. In the spectrum of **2**, there were several signals due to methyl and ketal carbons of the isopropylidene group. A distinct signal appearing at δ 105.21 was assignable to C-1 of the α-furanose ring because of its considerable downfield shift. In the spectrum of **3**, however, not only the isopropylidene signals but also the

Table II
Optical Rotations

sample	$[\alpha]_D$[a], deg	solvent
1	−27.9	chloroform
2	−23.1	chloroform
3	+32.9	water
4	−3.5	water
5	+4.5	water
6	+30.5	Me_2SO

[a] Concentration 1%.

α-furanose C-1 signal disappeared completely. Furthermore, the absorptions of the glucose residue appearing at δ 96.05 to 69.7 were rather complex, suggesting the presence of a mixture of α- and β-pyranose ring forms. The signals assignable to the C-1 and C-3 carbons of the pyranose ring were enlarged in the insert, together with the assignments. The α-/β-anomer ratio, calculated from their peak areas, was 0.46 ± 0.03:0.54 ± 0.03. The α-anomer content was found to be a little higher than that of D-glucose, the α-/β-anomer ratio of which was reported to be 0.373 ± 0.01:0.626 ± 0.01.[45] The difference probably arose from the substituent effect of the benzyl group. It is noticeable that the spectrum of **5** is very simple; there appeared no anomeric signal but only five peaks were observed in the region of δ 78–62. These absorptions are indicative of the formation of the open chain glucitol form. The acetylated product **6** also showed peak separation due to α- and β-pyranose forms; its C-1 signals were enlarged in the insert. The calculated α-/β-anomer ratio agreed with that of the glucose residue in **3**. It means that the acetylation of the glucose residue in **3** with acetic anhydride in pyridine proceeded without anomeric change, in the same way as the acetylation of D-glucose under similar conditions.

The specific rotations of **1** through **6** are summarized in Table II. The changes of the ring structures described above were qualitatively confirmed by the sign and magnitude of the rotations. Thus **1** and **2** exhibited negative rotations characteristic for the α-furanose form. Conversion to **3** resulted in the change of the sign. It was found that the mutarotation was completed during the deblocking procedure. The rotation of **3**, together with that of **6**, seems reasonable for the mixture of α- and β-pyranose ring forms suggested from the ^{13}C NMR data. The observed quite small rotations of **4** and **5** are also indicative of open chain forms.

The polymer **2** described in Table I was of high molecular weight. For example, **2** with an intrinsic viscosity of 0.68 (in benzene) had a molecular weight ($\bar{M}_n$) of 1.58 × 10^5 ($\overline{DP}_n$ = 420; $\bar{M}_w$ = 4.59 × 10^5; $\overline{DP}_w$ = 1220), which was estimated from the GPC retention time–molecular weight relationship derived for standard polystyrenes. As degradation of the polymer backbone was unlikely to happen during all the reactions attempted, the polymers **2** through **6** can be considered to have a similar degree of polymerization.

The polymers **3**, **4**, and **5** were soluble in water; the solubility in 100 mL of water was in the order **5** (~15 g) > **3** (~10 g) > **4** (~1 g). When aqueous solutions of the polymers were agitated, a mass of stable bubbles was formed. The aqueous solutions also could emulsify organic solvents such as chloroform and benzene. Table III summarized the intrinsic viscosities of **2**, **3**, and **5** derived from the same origin. Interestingly, the viscosities varied widely with polymer structures and solvents. Particularly, it is noteworthy that **3** and **5** exhibited remarkably small viscosities in water. In addition, their Huggins constants (k') were found to be nearly zero in water, suggesting that the polymers were in a tightly-coiled conformation in water. This characteristic conformation was also supported by the comparison of the ^1H and ^{13}C NMR spectra of **3** and **5** in deuterium oxide with those in Me_2SO-d_6 solutions. Significant broadening of the signals, especially of the phenyl signals, was observed in deuterium oxide rather than in Me_2SO-d_6 solution. This is an indication of the small mobility of the main chain and the intense stacking of the phenyl groups. These interesting findings prompted us to investigate the interaction of **3** with an organic solute in water.

Table III
Intrinsic Viscosities of Polymers[a]

polymer[b]	$[\eta]$ in benzene	$[\eta]$ in Me_2SO	$[\eta]$ in water
2	0.93	(0.35)[c]	
3		1.38	0.26
5		1.65	0.27

[a] At 25 °C. [b] The samples were derived from the number K24 in Table I. [c] At 40 °C.

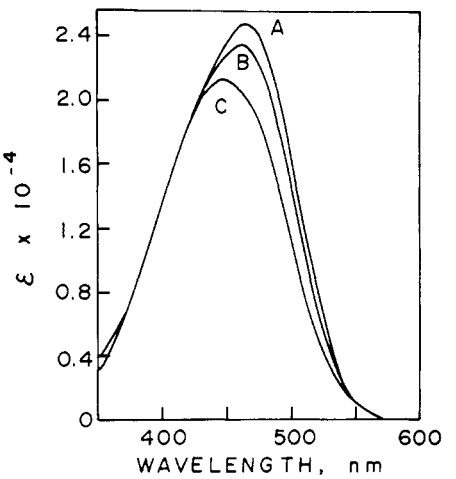

Figure 3. Absorption spectra of methyl orange: A, free methyl orange; B, methyl orange in the presence of 3; C, assumed for bound methyl orange; [MO] = 1 × 10^{-4} M; [3] = 5 × 10^{-3} M.

Binding of Methyl Orange to 3 in Water. Figure 3 shows the absorption spectrum of methyl orange (A) and that of a mixture of methyl orange and **3** (B) in phosphate buffer. It can be seen that the polymer induced a decrease in intensity as well as a blue shift of the main 464-nm methyl orange absorption band. In order to facilitate more quantitative treatment, difference spectra between a methyl orange reference solution in the absence of **3** and methyl orange sample solutions in the presence of various concentrations of **3** were taken and depicted in Figure 4. The spectra had minimums at 390 and 493 nm. The appearance of an isosbestic point at 362 nm may be significant in the following two respects. First, there exist two different species of free and bound methyl orange in the sample solution. Second, absorption of the polymer itself did not appear in the spectra obtained, and hence the difference optical density (ΔOD) can be expressed only in the terms of methyl orange by

$$\Delta OD = \{[MO]_B\epsilon_B + [MO]_F\epsilon_F\} - \{[MO]\epsilon_F\} \quad (1)$$

where $[MO]_B$, $[MO]_F$, and [MO] stand for the concentrations of the bound, the free, and the total methyl orange and ϵ_B and ϵ_F for the molar absorbances of the bound and the free methyl orange, respectively. As the concentration

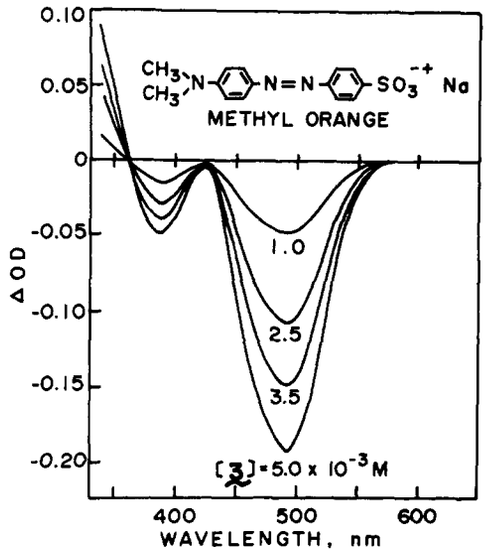

Figure 4. Difference absorption spectra of methyl orange in the presence of 3; [MO] = 1×10^{-4} M.

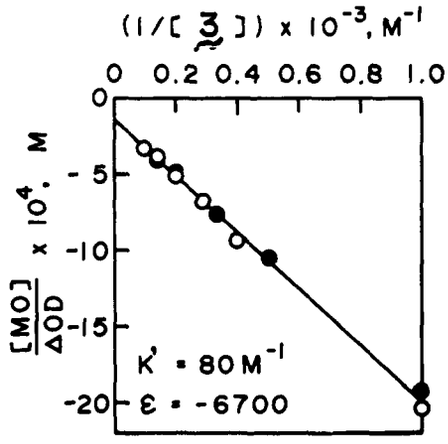

Figure 5. Benesi-Hildebrand relationship for binding of methyl orange to 3: (○) [MO] = 1×10^{-4} M; (●) [MO] = 0.5×10^{-4} M; ΔOD, the difference optical density at 493 nm.

of methyl orange in the sample solution was kept precisely equal to that in the reference, eq 2 was valid.

$$[MO] = [MO]_B + [MO]_F \quad (2)$$

Therefore, eq 1 may be reduced to a very simple form.

$$\Delta OD = [MO]_B(\epsilon_B - \epsilon_F) \quad (3)$$

In Figure 5 was plotted the Benesi-Hildebrand relationship, eq 4, using the ΔOD at 493 nm

$$\frac{[MO]}{\Delta OD} = \frac{1}{[3]}\frac{1}{K'\epsilon} + \frac{1}{\epsilon} \quad (4)$$

where ϵ is equal to $\epsilon_B - \epsilon_F$. The binding constant K' was calculated from the slope of the straight line to be 80 M^{-1}. The intercept gave a value of −6700 for $\epsilon_B - \epsilon_F$ at 493 nm, which led to $[MO]_B$ by eq 3 and then to $[MO]_F$ by eq 2. Since ϵ_F could be read in Figure 3A, ϵ_B at various wavelengths was calculated and plotted in Figure 3C. The assumed absorption spectrum of the methyl orange bound to 3 was found to have λ_{max} at 445 nm (ϵ_{max} = 21 300), which was blue shifted by about 20 nm from that of the free methyl orange (λ_{max} 464 nm; ϵ_{max} = 25 100).

Figure 6 shows the plots of eq 5, a rearranged form of

$$\frac{[3]}{[MO]_B} = \frac{1}{[MO]_F}\frac{1}{Kn} + \frac{1}{n} \quad (5)$$

the Langmuir isotherm suggested by Klotz.[34] The first

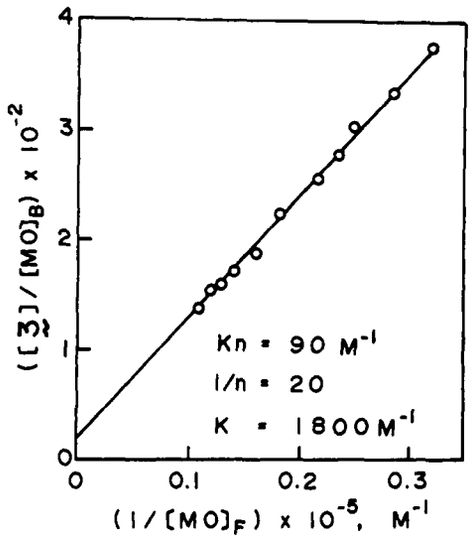

Figure 6. Klotz relationship for the binding of methyl orange to 3.

Table IV
Binding between Methyl Orange and Polymers

polymer	λ_{max} of bound methyl orange,[a] nm	$K^* \times 10^{-4}$, $(10^5$ g/L$)^{-1}$	ref
poly(vinyl-pyrrolidone)	470	2.7[b,d]	36
		1.79[b,d]	37
3	445	3.0[c,e]	*f*
bovine serum albumin	435	6.1[b,d]	36
poly(crown ether)	435	33.4[c,d]	40

[a] Free methyl orange, 464 nm. [b] Equilibrium dialysis. [c] Spectrophotometric method. [d] At 25 °C. [e] At room temperature. [f] This paper.

binding constant, Kn, was calculated to be 90 M^{-1}, which agreed closely with the binding constant obtained from the Benesi-Hildebrand relationship. The intercept $(1/n)$, which represents the minimum number of structural units required to bind a solute molecule, was 20. Therefore, the intrinsic binding constant K was calculated to be 1800 M^{-1} from $(Kn) \times (1/n)$. From these binding constants it was clearly deducible that methyl orange was bound to 3 strongly.

Various types of substances that can bind organic molecules have been well documented.[47] Binding in micelles and biomembranes is attributable to the formation of noncovalent assemblies of relatively small molecules that usually have long methylene chains and polar ionic groups. The inclusion cavity and the ion-binding property are responsible for the binding in cyclodextrins and crown ethers, respectively. In addition, some types of macromolecules are also known to bind organic solutes in aqueous solution. In many of these systems, introduction of charged groups into polymers as well as the cross-linking of polymers is reported to increase the binding ability. However, 3 is a nonionic polymer which carries neither charged group, nor long methylene chain, nor inclusion cavity, nor cross-linking. Nevertheless, 3 has a strong affinity for the organic solute.

Among nonionic macromolecules, poly(vinylpyrrolidone) is known to exhibit the most effective binding. Therefore, it is noteworthy that the Kn value of 3 was about four

times as large as that of poly(vinylpyrrolidone).[36,37] The constant Kn, however, depends upon the size of the specified unit and hence K^* converted in terms of 10^5 g of polymer is frequently used for comparison of different polymers in the literature. It is valid even from the comparison of the K^* values listed in Table IV that the binding of methyl orange to **3** was stronger than that of poly(vinylpyrrolidone).[36,37] Bovine serum albumin[36] and poly(crown ether)[40] were superior to **3**, although it would perhaps be unfair to compare the binding strength of **3** with these polymers possessing ionic or ion-binding character.

Table IV also shows the λ_{max} of bound methyl orange, which can be a measure of the polarity in the immediate vicinity of the bound species. Poly(vinylpyrrolidone) was supposed to bind methyl orange in a water-like hydrophiphic environment, since it caused very little red shift of the λ_{max}.[36] On the other hand, it was found that **3** induced the blue shift of methyl orange which was similar to those reported for bovine serum albumin[36] and poly(crown ether).[40] The shifts have been considered to reflect an apolar environment of the bound species.

It is evident that the sugar moeity of **3** has affinity for water whereas the vinylbenzyl residue avoids water. Actually, **3** was soluble in water, but the viscosity and NMR measurements suggested that the polymer sequence resisted spreading out into water to result in a tightly-coiled conformation. Furthermore, it is reasonable to assume that intramolecular aggregation of the vinylbenzyl residues occurred to form hydrophobic regions which were enclosed in hydrophilic surroundings of water-solvated sugar residues. The size of a hydrophobic region would depend upon various factors, one of which would be a steric requirement that one end of the amphiphile was covalently connected. It seems likely that one hydrophobic region was made up of about 20 repeating units which corresponded to the $1/n$ value obtained from the Klotz relationship. One may conclude that methyl orange was strongly bound to one of these hydrophobic regions of a polymer molecule in water.

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