Molecular Weight Distribution of Synthetic Stereoregular Polysaccharides. II. Tribenzyl Derivatives of (1→6)-α-Glucan, -Mannan, and -Galactan

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ABSTRACT: The previously developed method for determining sedimentation coefficient distributions (SCD) was applied to the characterization of molecular weight distributions (MWD) of novel, synthetic, stereoregular, and high molecular weight (1→6)-α-glucans, -mannans, and -galactans, in the form of their tribenzyl derivatives. The results indicated a general applicability of the method, with some limitations. Sequential corrections for pressure, diffusion, and concentration effects were found to be necessary. The resulting MWD's were checked by independent molecular weight determinations and were found to be within acceptable error limits. The values of the Flory constant β necessary for the conversion of SCD to MWD were determined and indicated the glucan to be a more flexible chain than either the mannan or galactan. This was in keeping with other molecular parameters of these polysaccharides and their solubilities. The observed MWD's were not inconsistent with the "most probable" mechanism of synthesis.

In the previous communication of this series, we reported on the successful application of the sedimentation velocity method, complete with corrections for pressure, diffusion, and concentration effects, to the analysis of molecular weight polydispersity of synthetic tribenzylglucan.(1) The analysis revealed a favorable comparison of the polydispersity index $\bar{M}_w/\bar{M}_n$ obtained both from the sedimentation method and from independent molecular weight determinations. However, conversion of the sedimentation coefficient distribution (SCD) to the corresponding molecular weight distribution (MWD) using the accepted average value of the Flory constant β resulted in lower average molecular weights than obtained independently from osmometry and light scattering.

This study has now been extended to cover not only the (1→6)-α-glucan, but the (1→6)-α-mannan and (1→6)-α-galactan as well, in the form of their tribenzyl derivatives. The above three types of polysaccharides are novel, completely linear and stereoregular polymers of considerable biomedical importance, and can only be obtained synthetically by methods developed at this laboratory.(2-11) The MWD of these polysaccharides is of interest for several reasons—to obtain information leading toward understanding the mechanism of synthesis, to help explain solubility differences, and to complement the solid-state conformational studies presently underway on these polymers. In addition, answers to questions concerning further applicability and limitations of the sedimentation velocity method, especially to samples of higher polydispersity, and the proper values of the Flory constant β were sought from this work.

Experimental Section

Samples. Four different (1→6)-α-tribenzylgalactans and two (1→6)-α-tribenzylmannans, previously synthesized, were analyzed. A third sample of mannan was incompletely analyzed. The sample codes and pertinent physical properties are listed in Table I, along with the data for two (1→6)-α-tribenzylglucans which were studied earlier.(1) The latter two polymers were not reanalyzed experimentally, the previously obtained data were used for the calculations.

Sedimentation Experiments. All sedimentation experiments were performed in a Spinco Model E analytical ultracentrifuge at 8° as previously described.(1) The measured boundary gradient curves were converted to the corrected SCD ($g(S_0)$ *vs.* S_0) by successively removing the pressure, diffusion, and concentration effects, in that order and in the manner also previously described.(1)

Briefly, pressure corrected $g(S)$ *vs.* S curves were obtained from

$$g(S) = \frac{1}{A_0}\left(\frac{\mathrm{d}n}{\mathrm{d}r}\right)\left(\frac{r}{r_0}\right)^2 r\omega^2 t[1 - m_a(y - 1)]^2 \qquad (1)$$

and

$$S = \frac{1}{2(m_a + 1)\omega^2 t} \ln \frac{y}{1 - m_a(y - 1)} \qquad (2)$$

where A_0 is the initial area under the boundary gradient curve, n is the refractive index of the solution, r and r_0 are the distances from the center of revolution to, respectively, a specified point in the ultracentrifuge cell and the meniscus, ω is the angular velocity of revolution, t is the time from the start of sedimentation, m_a is the pressure correction parameter, and $y = (r/r_0)^2$.

Diffusion correction was effected by extrapolation of the individual $g(S)$ *vs.* S curves to infinite time. Concentration correction was made in two ways, using equations

$$S = S_0(1 - kc) \qquad (3)$$

$$1/S = (1/S_0)(1 + k'c) \qquad (4)$$

where k and k' are empirical constants and c is the concentration.

In some cases it was found that the fully corrected curves of $g(S_0)$ *vs.* S_0 showed discontinuities at low values of $g(S_0)$, probably due to experimental error. In these cases a continuous curve was fitted to experimental points using either a Gaussian or a skewed Gaussian function. The fitting was accomplished with the aid of a least-squares curve resolution computer program.(12) In cases where the skewed Gaussian fit was best it was observed that a physically unreasonable extended tail resulted on the high S_0 side of the distribution due to the mathematical form of the asymmetric Gaussian function. In such cases the extended tail was brought to $g(S_0) = 0$ at a point consistent with the slope of the $g(S_0)$ *vs.* S_0 function at intermediate values of $g(S_0)$ on the high S_0 side of the distribution curve.

The calculations were made on a CDC 3200 computer equipped with a plotter using a previously developed program.(13)

Conversion of SCD to MWD. The molecular weight distribution function $f(M)$ was calculated from the corresponding SCD using the equations of Wales and Rehfeld(14)

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(1) J.-P. Merle and A. Sarko, *Macromolecules*, **5**, 132 (1972).
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(14) M. Wales and S. J. Rehfeld, *J. Polym. Sci.*, **62**, 179 (1962).

Table I
Sample Characteristics

Sample No.	$\overline{M}_n$ (Osmometry)	$\overline{M}_w$ (Light Scattering)	$\overline{M}_w/\overline{M}_n$	$[\eta]_{\text{benzene}}^{8°}$	Mark-Houwink Exponent a	Partial Sp Vol, $\bar{v}$ (cm^3/g)	dn/dc
LGB 38[a]	1.29×10^5	1.86×10^5	1.4 (±0.2)	0.086	0.59	0.74	0.085 (±0.007)
GL 21[a]	3.47×10^5	8.89×10^5	2.5 (±0.4)	0.225	0.59	0.74	0.085 (±0.007)
TBGA 31	4.55×10^4	9.22×10^4	2.0 (±0.3)	0.055	0.76	0.85	0.080 (±0.007)
TBGA 30	8.85×10^4	1.89×10^5	2.1 (±0.4)	0.095	0.76	0.85	0.080 (±0.007)
TBGA 28	1.20×10^5	2.41×10^5	2.0 (±0.3)	0.112	0.76	0.85	0.080 (±0.007)
TBGA 25	2.14×10^5	3.88×10^5	1.9 (±0.3)	0.195	0.76	0.85	0.080 (±0.007)
TBM 18[b]	1.00×10^5	2.90×10^5	2.9 (±0.5)	0.120	0.76	0.85	0.077 (±0.007)
TBM 21	2.24×10^5	6.85×10^5	3.0 (±0.5)	0.255	0.76	0.85	0.077 (±0.007)
TBM 25	1.97×10^5	5.24×10^5	2.7 (±0.5)	0.230	0.76	0.85	0.077 (±0.007)

[a] From ref 1. [b] Not analyzed by sedimentation velocity methods.

$$f(M) = \frac{2 - a}{3} \frac{g(S_0)}{M_{sv}} \frac{S_{0,\max}^{3/2}}{S_0^{1+a/2-a}} \left[\int_0^\infty S_0^{3a/2-a} g(S_0) \mathrm{d}S_0 \right]^{1/2} \tag{5}$$

$$M = \frac{S_0^{3/2-a} M_{sv}}{S_{0,\max}^{3/2} \left[\int_0^\infty S_0^{3a/2-a} g(S_0) \mathrm{d}S_0 \right]^{1/2}} \tag{6}$$

where $S_{0,\max}$ is the sedimentation coefficient at the maximum of the $g(S_0)$ distribution, a is the Mark–Houwink exponent, and M_{sv} is calculated using

$$M_{sv} = \left[\frac{\eta_0 N}{(1 - \bar{v}\rho)\beta} \right]^{3/2} [\eta]^{1/2} S_{0,\max}^{3/2} \tag{7}$$

where η_0 is the solvent viscosity, $\bar{v}$ is the partial specific volume of the polymer, ρ is the solvent density, $[\eta]$ is the intrinsic viscosity of the macromolecule, N is the Avogadro number, and $\beta = \Phi^{1/3} P^{-1}$ as defined by Flory.[15]

Similarly, the weight- and number-average molecular weights and the polydispersity index were calculated using the equations

$$\overline{M}_n = \frac{1}{\int_0^\infty 1/M f(M) \mathrm{d}M} = \frac{M_{sv}}{S_{0,\max}^{3/2}} \frac{1}{\int_0^\infty S_0^{-3/2-a} g(S_0) \mathrm{d}S_0 \left[\int_0^\infty S_0^{3a/2-a} g(S_0) \mathrm{d}S_0 \right]^{1/2}} \tag{8}$$

$$\overline{M}_w = \int_0^\infty f(M) \mathrm{d}M = \frac{M_{sv}}{S_{0,\max}^{3/2}} \frac{\int_0^\infty S_0^{3/2-a} g(S_0) \mathrm{d}S_0}{\left[\int_0^\infty S_0^{3a/2-a} g(S_0) \mathrm{d}S_0 \right]^{1/2}} \tag{9}$$

$$\overline{M}_w / \overline{M}_n = \int_0^\infty S_0^{3/2-a} g(S_0) \mathrm{d}S_0 \int_0^\infty S_0^{-3/2-a} g(S_0) \mathrm{d}S_0 \tag{10}$$

Molecular Weight Determinations. Average molecular weights of all samples were measured both by osmometry in toluene solutions and light scattering in benzene solutions. Osmometric measurements were done with a Hewlett-Packard rapid membrane osmometer at concentrations of 0.17–1.76 g/l. and light-scattering measurements were done with an Aminco light-scattering photometer at concentrations of 0.1–2.0 g/l. The solutions for light scattering were purified by filtration through Millipore filters with a pore sizes of 0.45 μ.

Miscellaneous Measurements. Intrinsic viscosities in benzene solutions at 8° were determined for all samples, using an Ubbelohde viscometer at concentrations of 0.015–0.22 g/dl. The Mark–Houwink exponent was calculated from a log-log plot of $[\eta]$ *vs.* M_w. Measurements of dn/dc were done with a Phoenix differential refractometer at concentrations of 0.1–1.6 g/l. in benzene solutions. Specific volumes of the samples were calculated from measurements of the density of solution at varying concentrations using the following equation[14]

$$\bar{v}_{\text{polymer}} = \bar{v}_{\text{soln}} + (1 - x)(\mathrm{d}\bar{v}_{\text{soln}}/\mathrm{d}x) \tag{11}$$

where x is the weight fraction of polymer. These measurements were made at concentrations of 0.07–0.36 wt %.

Results and Discussion

Methods. As previously observed, pressure and diffusion effects were found to be significant in all cases studied. However, no systematic differences in the magnitudes of these effects between the three types of polysaccharides were found even in cases of higher polydispersities. Experimental scatter still remained a problem, undoubtedly due to the use of the schlieren method, and manifesting itself in extreme cases by producing a negative pressure correction parameter (*i.e.*, an apparent lower pressure at the bottom of the centrifuge cell than at the top). The diffusion extrapolations were less troublesome, except at the tails of the distribution where experimental error was again more prominent.

In our previous work with the (1→6)-α-tribenzylglucan, the removal of the concentration influence was more effective using the $1/S$ extrapolation (eq 4) rather than the S extrapolation (eq 3). The measure of effectiveness was based on the resulting values of $\overline{M}_w/\overline{M}_n$ and the linearity of the plots. In the present work, both methods were again used and the degree of effectiveness could in addition be based on the conversion of SCD to MWD. An attempt was also made to obtain S_0 through a quadratic extrapolation

$$S = S_0(1 - k_1 c + k_2 c^2) \tag{12}$$

However, the distributions resulting from this procedure were usually nearly identical with those resulting from the $1/S$ *vs.* c extrapolation and in some cases where experimental scatter was higher than usual, the results were unreasonable. In most cases, using the criteria outlined above, the $1/S$ *vs.* c extrapolation proved to be superior to the S *vs.* c extrapolation. In two cases, those involving the mannans (samples TBM 21 and TBM 25), the indices obtained from both methods of extrapolation were nearly identical; however, based on the fact that a more reasonable value of the constant β resulted when the $1/S$ *vs.* c extrapolation was used (see below) the latter method was again preferred.

The three extrapolations to obtain the SCD corrected for pressure, diffusion and concentration effects are each subject to certain conditions. When extrapolating $\bar{S}$ *vs.* $\bar{y} - 1$ in determining the pressure correction parameter

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[15] P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

Table II
Molecular Weights, Polydispersity Index, and Average Constant β Evaluated from the Sedimentation Analysis

Sample No.	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	β
LGB 38	1.15×10^5	1.80×10^5	1.6	$1.93 (\pm 0.3) \times 10^6$
GL 21	3.70×10^5	8.67×10^5	2.3	$1.93 (\pm 0.3) \times 10^6$
TBGA 31	3.61×10^4	8.82×10^4	2.4	$2.45 (\pm 0.3) \times 10^6$
TBGA 30	1.28×10^5	2.33×10^5	1.8	$2.45 (\pm 0.3) \times 10^6$
TBGA 28	1.10×10^5	3.10×10^5	2.8	$2.45 (\pm 0.3) \times 10^6$
TBGA 25	1.77×10^5	3.38×10^5	1.9	$2.45 (\pm 0.3) \times 10^6$
TBM 21	2.72×10^5	7.77×10^5	2.9	$2.55 (\pm 0.23) \times 10^6$
TBM 25	1.83×10^5	5.80×10^5	3.2	$2.55 (\pm 0.23) \times 10^6$

m_a (*cf.* eq 9 in ref 1), the product $m_a(y - 1)$ must be small compared to unity. When extrapolating to remove diffusion dependence, Gosting has shown that the range of experimental times t must be limited for the extrapolation to be linear.[16] Williams *et al.* have found that, practically speaking, the concentration extrapolations should be made using concentrations at which the ratio S_0/S falls in the range 0.9–1.1.[17] In this investigation, the first two conditions were met. In some cases the ratio S_0/S was out of the desired range at large values of the sedimentation coefficients in the SCD, without, however, causing apparent harm.

The method was, in addition, checked by analyzing two standard polystyrenes (National Bureau of Standards Polystyrene No. 705 $\bar{M}_n = 1.71 \times 10^5$, $\bar{M}_w = 1.79 \times 10^5$, $\bar{M}_w/\bar{M}_n = 1.07$ and Polystyrene No. 706, $\bar{M}_w = 2.88 \times 10^5$, $\bar{M}_w/\bar{M}_n = 2.1$) in parallel with the tribenzyl polysaccharides. The narrow distribution material gave acceptable results, with the average molecular weights approximately 15% lower than those quoted above, using $\beta = 2.3 \times 10^6$.[15] The polydispersity index was also acceptable at 1.14. On the other hand, the broad distribution sample failed to produce correct results. The reason for the failure probably stemmed from the type of distribution which was severely skewed toward smaller molecular weights and peaked at a large molecular weight.

Conversion to MWD and the Constant β. As previously noted, the average values of $\beta = 2.5 \times 10^6$, when applied to the calculation of $\bar{M}_n$ and $\bar{M}_w$ of the glucans according to eq 8 and 9, resulted in average molecular weights that were approximately 30% lower than those determined independently by osmometry and light scattering.

An attempt to evaluate applicable values of the constant β for the three types of polysaccharides was made in the following manner. The values of $\bar{M}_n$ and $\bar{M}_w$ determined by osmometry and light scattering were substituted into eq 7, 8, and 9 which were then solved for β. The average values of β thus obtained along with $\bar{M}_n$ and $\bar{M}_w$ calculated using these values are shown in Table II. It is apparent that the three types of polysaccharides fall into two classes as regards the constant β—the tribenzylmannan and galactan in one class with β very close to the average $\beta = 2.5(\pm 0.1) \times 10^6$ as reported by Flory,[15] and the tribenzylglucan in the other class with a considerably lower value. Although this is somewhat puzzling, the trend is consistent with other solution parameters—the Mark-Houwink constant a and the partial specific volume $\bar{v}$, as is evident from Table I. Both a and $\bar{v}$ for the glucan are considerably lower than the corresponding values for the mannan and galactan which are identical. This difference may indicate that the glucan is a more flexible chain than either the mannan or the galactan. From the point of view of the chemical structure of these polysaccharides this is a reasonable assumption, *viz.*, all three benzyl-substituted hydroxyl groups of the glucan monomer residue are equatorial while in the mannan the C-2 substituent is axial and similarly, in the galactan the C-4 substituent is axial. In either case, the axial substituent is expected to reduce the reasonably free rotation about the three bonds linking successive monomer residues. This expectation is substantiated by differences in solubility observed for the three parent (unsubstituted) polysaccharides. The glucan is much more soluble than either the mannan or the galactan, with the latter being the least soluble of the three polymers. Apparently, the value of β reflects the degree of flexibility of the polysaccharide chain; this is substantiated by the higher than average value of $\beta = 2.7 \times 10^6$ observed for cellulose acetate,[15] a less flexible polysaccharide than most.

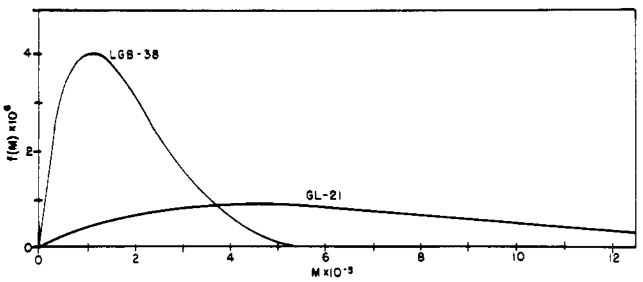

Figure 1. Molecular weight distributions of the tribenzylglucans.

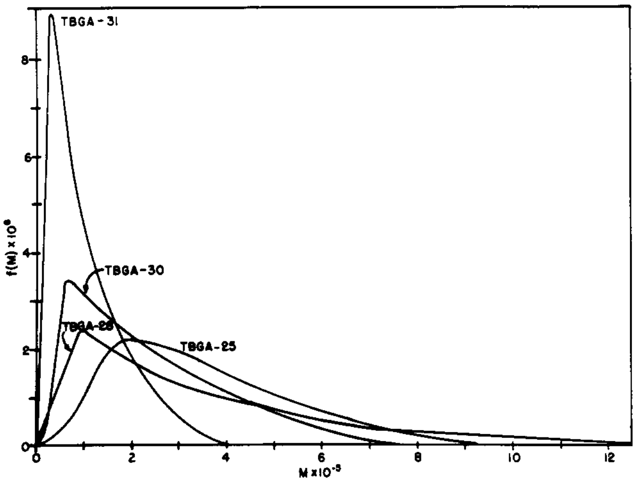

Figure 2. Molecular weight distributions of the tribenzylgalactans.

Molecular Weight Distributions. The MWD's calculated from the corresponding SCDs using values of β listed in Table II are shown in Figures 1–3. It is apparent that the synthesis of the three classes of tribenzyl polysaccharides produced MWD's that were all of the same type —with peaks in the distribution curve occurring at low molecular weights. There was little difference in the distributions between tribenzylmannans and -galactans, although the former were considerably more polydisperse than the latter. On the other hand, the distributions of the two tribenzylglucans were considerably different. It appears that in the case of the glucans the increase in the degree of polymerization was accompanied by a large increase in the polydispersity, something that was not observed with mannans and galactans. (Two additional tribenzylglucans intermediate in the degree of polymerization to those shown here tended to substantiate these re-

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(16) L. J. Gosting, *J. Amer. Chem. Soc.*, 74, 1548 (1952).

(17) J. W. Williams, K. E. Van Holde, R. L. Baldwin, and H. Fujita, *Chem. Rev.*, 58 715 (1958).

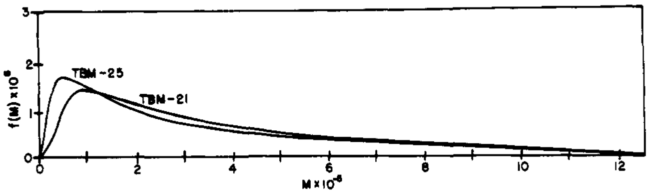

Figure 3. Molecular weight distributions of the tribenzylmannans.

sults. Their results are not shown because independent molecular weight measurements for them were not made owing to lack of sufficient sample.)

Little can be said at the present time concerning the bearing of the observed MWD's on the mechanism of synthesis of these polysaccharides, simply because kinetic studies have not been done on these systems. However, some observations can be made, particularly when comparing the mannans and galactans.

First, regarding the relative rates of propagation and initiation of these syntheses, it is likely that they must be at least comparable, because if the rate of propagation were much lower than the rate of initiation, the MWD would have been considerably narrower.

Jordan and Mathieson have calculated distribution equations for cationic polymerizations assuming a stationary state, and for different processes involving spontaneous termination, solvent transfer or monomer transfer.[18] For reactions with spontaneous termination or solvent transfer which have gone substantially to completion, their calculations predict curves of the type observed with mannans and galactans, with a shifting of the maximum in the MWD toward smaller molecular weights with increasing degrees of completion. When monomer transfer predominates, the MWD should resemble that of GL-21 and it should not be affected by the degree of completion of the reaction.

Mathieson, and Pepper and Burton have further shown that if monomer transfer is dominant, the distribution should be of the "most probable" type (with $\bar{M}_w/\bar{M}_n = 2$) at any conversion.[19,20]

Table III lists the molecular weights at the peak of the MWD, average polydispersity indices (obtained by averaging the two results obtained from the sedimentation analysis and independent molecular weight determinations) and the per cent conversions for the polymers of this study. As can be seen in the galactan series, the one polymer that has not reached a high degree of conversion (TBGA 25) shows a peak in the MWD which is considerably to the right of the other samples. On the other hand, in the same series of samples the polydispersity index remains near 2, regardless of the degree of conversion. These observations may indicate that for the galactans the "most probable" distribution is indeed in effect and that termination is largely spontaneous or by solvent (or impurity) transfer, with monomer or gegenion transfer not present. By the same token, the considerably larger polydispersity index of the mannans may indicate a more dominant role for monomer or gegenion transfer. On the other hand, it is also known that mannans polymerize at a faster rate than the galactans, which may or may not be important in this comparison.[5]

Table III
Peak Molecular Weights of the MWD's, Average Polydispersity Index, and Per Cent Conversion

Sample No.	Peak Mol Wt	Av $\bar{M}_w/\bar{M}_n$	% Conversion[21]
LGB 38	1.1×10^5	1.5	94
GL 21	4.3×10^5	2.4	85
TBGA 31	3.0×10^4	2.2	72
TBGA 30	6.5×10^4	1.95	91
TBGA 28	9.0×10^4	2.4	76
TBGA 25	2.0×10^5	1.9	42
TBM 18		2.9[a]	76
TBM 21	8.5×10^4	2.95	85
TBM 25	4.0×10^4	2.95	90

[a] From osmometry and light scattering only.

The results for the two glucans do not seem to fit the same pattern. Other glucans for which only incomplete data are available exhibited the same type of variability as shown by the present two samples. It is possible that variability in reaction conditions was more important to the synthesis of glucans in influencing the MWD than it was for the mannans and galactans.[21]

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(18) D. O. Jordan and A. R. Mathieson, *J. Chem. Soc.*, 2358 (1952).

(19) A. R. Mathieson, quoted in "The Chemistry of Cationic Polymerization," P. H. Plesch, Ed., MacMillan, New York, N. Y., 1963, p 298.

(20) D. C. Pepper and R. E. Burton, *Proc. Roy. Soc., Ser. A*, **263**, 58 (1961).

(21) C. Schuerch, private communication.