Carbohydrate Polymers 32 (1997) 17-22
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0144-8617/97/$17.00 + .00

ELSEVIER
PII: S0144-8617(96)00127-0

Physical and structural properties of barley $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions

C. Gómez[a]*, A. Navarro[a], P. Manzanares[a], A. Horta[b] & J. V. Carbonell[c]

[a] *Asociación de Investigación de Cerveza y Malta (INVESCEMA). P.O. Box 73, 46100-Burjassot, Valencia, Spain*
[b] *Departamento de Ciencias y Técnicas Fisicoquímicas, Facultad de Ciencias, Universidad a Distancia (UNED), C/Senda del Rey s/n, 28040 Madrid, Spain*
[c] *Instituto de Agroquímica y Tecnología de Alimentos (IATA, CSIC), P.O. Box 73, 46100-Burjassot, Valencia, Spain*

(Received 24 January 1996; revised version received 9 August 1996; accepted 23 August 1996)

Intrinsic viscosities and molecular weights of $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan samples, covering a range of 9×10^3–6×10^5 daltons, have been used to determine their conformational parameters and molecular dimensions.

The chain of β-glucan is satisfactorily modelled by a partially stiff worm-like cylinder, with persistence length 3.5–3.8 nm and cross-section diameter 0.45 nm. This corresponds to an average of about four $\beta(1\rightarrow3)$ links per statistical segments. The characteristic ratio, C_∞, calculated from this worm-like description of the macromolecule, gave a value of 13–14, in good agreement with the theoretical calculations. © 1997 Elsevier Science Ltd

INTRODUCTION

The major component in a barley endosperm cell-wall is $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan, which accounts for approximately 75% of total cell-wall carbohydrate, the rest being protein and pentosans (Fincher, 1975). This polysaccharide, for short referred to as β-glucan, can be mainly considered as a linear chain of β-$(1\rightarrow3)$-linked cellotriosyl and cellotetraosyl units arranged randomly, containing about 70% β-$(1\rightarrow4)$ and 30% β-$(1\rightarrow3)$ links (Igarashi & Sakurai, 1966; Wood *et al.*, 1994). However, longer blocks of up to 14 adjacent $\beta(1\rightarrow4)$-linkages exist in relative low frequency in the barley β-glucan molecule, whereas the existence of contiguous β-$(1\rightarrow3)$-linkages has not been detected by the latest studies (Edney *et al.*, 1991; Izawa *et al.*, 1993). The erratic spacing of the β-$(1\rightarrow3)$-linkages in the molecule and its higher flexibility than the β-$(1\rightarrow4)$-linkages (Sudaralingam, 1968) confer an irregular shape to the molecule, consistent with its solubility in water. Figure 1 shows a scheme of the $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan molecule.

$(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan has been extensively studied in brewing, since an excess of its content can cause several problems, such as slow filtration of worts and beers, decreased brewhouse yield and formation of haze and precipitates in stored beers (Bamforth, 1982). These problems depend not only on the final β-glucan content, but also on the molecular weight and chain conformation of these polymers. Otherwise, β-glucans are constituents of dietary fibre (McIntosh *et al.*, 1991) and have potential applications as thickening agents in the food industry.

Only a few systematic studies, focused on elucidating the structure and conformation of β-glucan molecules, have been published in Forrest & Wainwright (1977); Woodward *et al.* (1983); Buliga *et al.* (1986); Vårum *et al.* (1991); Wood *et al.* (1994), but the results still show some discrepancies. There is disagreement in the orders of magnitude among the molecular weights reported in the literature and also important differences in the viscosities of β-glucan solutions. These discordances had, up to now, been attributed to differences in sample sources, extraction conditions and measurement techniques.

In the present work, we have used a wide range of β-glucan samples (from 9×10^3 to 6×10^5 daltons) and determined the viscosity of their respective solutions in water and beer, by means of a capillary viscometer. From these results and from the light-scattering measurements of molecular weights of the same samples (Gómez *et al.*, 1996), conformational parameters of the β-glucan molecules were deduced and the results compared with the published data.

---

*Author to whom correspondence should be addressed.

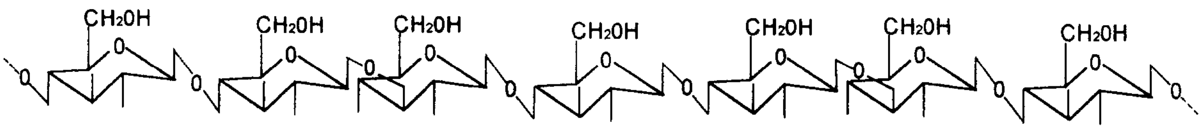

Fig. 1. Structure of barley (1→3),(1→4)-β-D-glucan.

MATERIALS AND METHODS

β-Glucan samples

Table 1 summarises the characteristics of β-glucan samples used in these experiments. The preparation and properties of these samples were described by Gómez *et al.* (1996) in the first part of this work. The intrinsic viscosity of β-glucan samples is obtained as described below.

For measurements in aqueous solutions, the range of concentrations, *c*, varied according to the molecular weight of the sample: from 0.15 to 0.50 g/dl for the lowest molecular weight (sample N), to 0.01–0.15 g/dl for the highest (sample A).

Beers of high β-glucan content (0.01–0.05 g/dl) were prepared by mixing eight parts of commercial beer (5.5% (v/v) alcohol) with two parts of an aqueous solution containing up to 5 g/l of β-glucan, in order to obtain a kind of beer having about 4.4% (v/v) alcohol. Commercial beer, free of β-glucanase activity as detected by the fluorimetric calcofluor—F.I.A. method (Navarro *et al.*, 1995), was previously degassed by sonication under vacuum and checked for its endogenous β-glucan content and average molecular weight (Manzanares *et al.*, 1993). The final samples retained, besides the added β-glucan, 0.008 g/dl of endogenous β-glucan.

Viscosity measurements

Relative viscosities ($\eta_r = \eta/\eta_0$, with η the viscosity of solution and η_0 that of the solvent) were measured at 25°C (±0.1°C) in an automated viscometer (CD 15 Lauda), fitted with an Ubbelohde tube (Schott-Geräte, type no. 531 13) and a falling time recorder (VB 12 Lauda). The limiting viscosity number (intrinsic viscosity $[\eta]$) was obtained by extrapolation to infinite dilution as defined by the Huggins ($\eta_{sp}/c = [\eta] + k' [\eta]^2 c$, with $\eta_{sp} = (\eta - \eta_0)/\eta_0$) or Kraemer equations ($(\ln \eta_r)/c = [\eta] + k'' [\eta]^2 c$). The possible influence of shear rate on the extrapolated intrinsic viscosity was checked by repeating the measurements for the sample G in another viscometric tube having a solvent flow time twice that of the tube routinely used. The result obtained for $[\eta]$ was the same in the two viscometers, within the experimental error.

RESULTS AND DISCUSSION

Viscosity equation

Figure 2 shows the Huggins and Kraemer extrapolations applied to the β-glucan samples A and N dissolved in water, which correspond to the highest and the lowest molecular weight samples (Table 1).

The intrinsic viscosity values deduced from Huggins and Kraemer plots are very close in all samples studied, but according to Kamide and Saito (1989) we can obtain more accurate $[\eta]$ values with the Kraemer equation, since the slope of this plot is smaller than that of Huggins. In addition, we have observed that the range of linearity is wider for the Kraemer plots.

Figure 3 shows the relationship between the relative viscosities of all aqueous β-glucan samples analysed as a function of their respective concentrations. The continuous lines were drawn using the values deduced by the Kraemer equations as fitting parameters. Similar beha-

Table 1. Characteristics of the β-glucan samples

Sample	Intrinsic viscosity (dl/g), in water	Intrinsic viscosity (dl/g), in beer	$M_n (\times 10^{-3})$	$M_w (\times 10^{-3})$	M_w/M_n
A	5.2	n.d.	482	573	1.18
B	4.6	n.d.	308	456	1.48
C	2.8	n.d.	197	278	1.41
E	3.0	n.d.	186	235	1.26
F	2.7	n.d.	179	231	1.30
G	2.7	2.90	164	231	1.41
I	1.77	1.80	78	105	1.35
J	1.38	1.61	69	106	1.54
L	0.73	0.74	31	36	1.16
M	0.76	n.d.	24	27	1.12
N	0.28	0.25	8.4	9.2	1.10

n.d. = not determined.

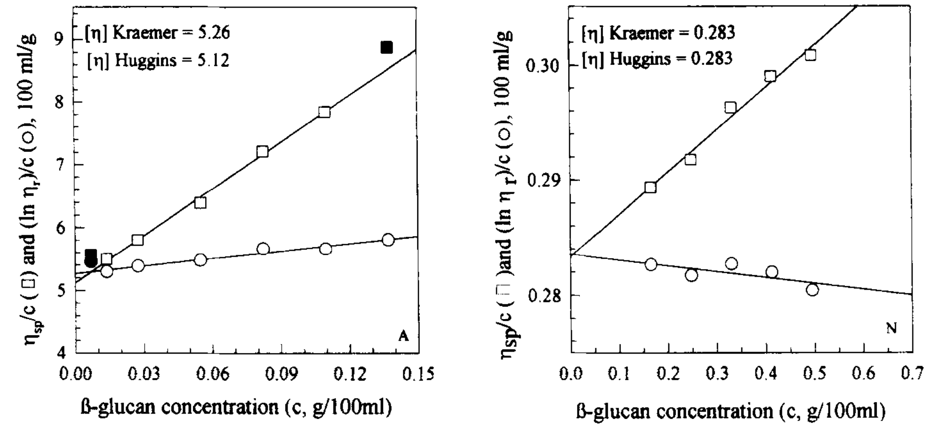

Fig. 2. Concentration dependence of η_{sp}/c and (ln η_r)/c for (1→3),(1→4)-β-D-glucans in aqueous solutions (black symbols were not used in the regression fitting).

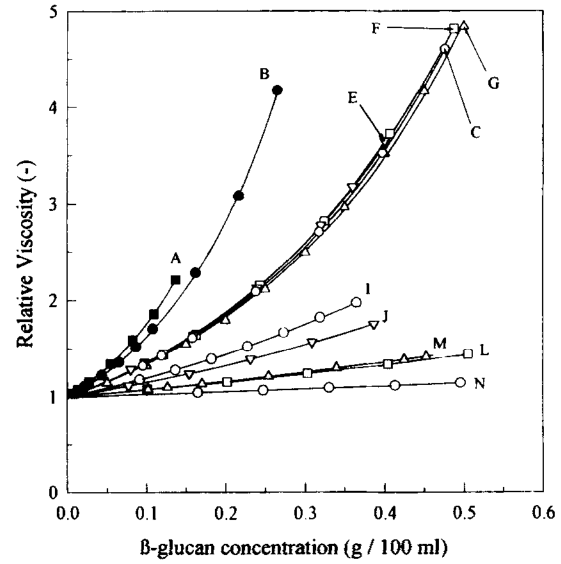

Fig. 3. Viscosity of aqueous β-glucan solutions (symbols correspond to experimental points and continuous lines to the fit using the Kraemer equation).

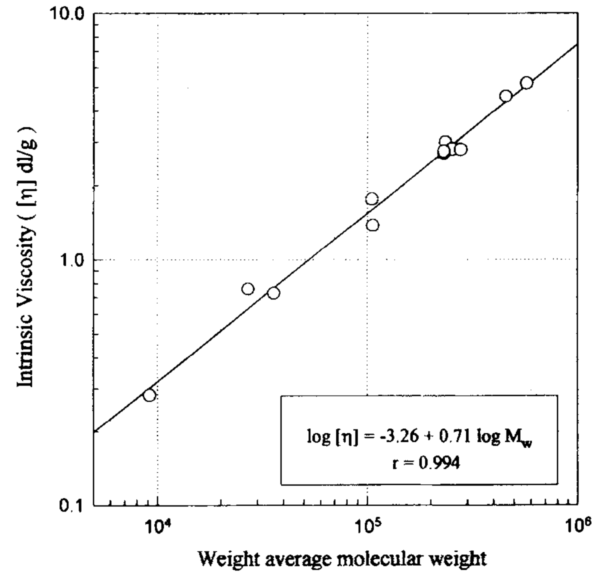

Fig. 4. The Mark–Houwink–Sakurada equation for aqueous β-glucan solutions.

viour was shown by β-glucan samples dissolved in beer.

Table 1 shows that the intrinsic viscosity of β-glucans in water solution and in beer were practically coincident. The small differences can be attributed to experimental errors, being more concordant with the data of aqueous solutions, due to their wider range of β-glucan concentrations and to the difficulty of a complete degasification of beers. Consequently, the following data and comments are valid for β-glucan molecules dissolved in water, as well as in beer.

From these results and from the weight average molecular weights of the different samples, the Mark–Houwink–Sakurada equation, $[\eta] = k\ M^a$, was deduced. The experimental data and the fitted function are shown in Fig. 4. The results of log[η] vs log M_w show good linearity in the whole range of molecular weight. The scaling exponent, $a = 0.71$ is well above the random coil value. Pure water is not a good solvent for β-glucans, so excluded volume should not be the main reason for such a high exponent, rather it should be chain stiffness, as we shall discuss below. Other determinations of the viscosity equations of β-glucan, in different solvent conditions and for different ranges of molecular weight, also show exponents very close to the present one. Thus, Vårum *et al.* (1991) obtained $[\eta] = 0.00067\,M_n^{0.75}$ in 0.1 M NaCl for the range $M_n = 2.0$–7.8×10^4, and $[\eta] = 0.00050\,M_n^{0.75}$ in 1 M LiI for the range $M_n = 6.3$–33×10^4. Also, Grimm *et al.* (1995) obtained a scaling exponent of 0.72, in maltose solutions, for the range $M_w = 2.8$–12×10^6 (all of these were at 20°C).

Macromolecular dimensions

The results of intrinsic viscosity and molecular weight were used to characterise chain stiffness in two ways: one by obtaining the unperturbed dimensions of the chain; another by obtaining the statistical segment length and the cross-sectional diameter of the macromolecule. In one case, the chain is modelled as a coil, in the other as a worm-like cylinder.

When the chain is treated as a coil, the relevant magnitude for dimensions is the mean square end-to-end distance of the coil, $\langle R^2 \rangle$. Its unperturbed value, $\langle R^2 \rangle_0$, occurs when the coil is not expanded by the excluded volume effect (θ conditions). The characteristic ratio, C_∞, is defined as the ratio of the real $\langle R^2 \rangle_0$ to the value it would have got if the units of the chain were freely-jointed:

$$C_\infty = \langle R^2 \rangle_0 / Nb^2$$

N being the number of units of length, b. To obtain the unperturbed dimensions from intrinsic viscosity data the standard Stockmayer–Fixman plot was applied ($[\eta]M^{-1/2}$ vs $M^{1/2}$). The intercept of this plot gives the unperturbed dimensions, expressed as K_θ. This K_θ is related to $\langle R^2 \rangle_0$ through $K_\theta = \Phi(\langle R^2 \rangle_0 / M)^{3/2}$, where Φ is Flory's viscosity constant ($\Phi = 2.6 \times 10^{21}$ mol^{-1}).

The Stockmayer–Fixman plot is shown in Fig. 5. For comparison, this graph plots, besides these results, others from the literature. Although the points corresponding to other authors are well above our experimental points, the extrapolated K_θs do not differ much. For example, our results using M_W yield (in dl g$^{-3/2}$ mol$^{1/2}$) $K_\theta = 3.1 \times 10^{-3}$. The results of Vårum *et al.* (1992) with M_n (in the range where they are linear) give $K_\theta = 4.7 \times 10^{-3}$. If we use M_n with our results, then the extrapolated value is $K_\theta = 3.3 \times 10^{-3}$. Taking the results reported by Buliga et al. (1986), a value of $K_\theta = 4.4 \times 10^{-3}$ can be deduced.

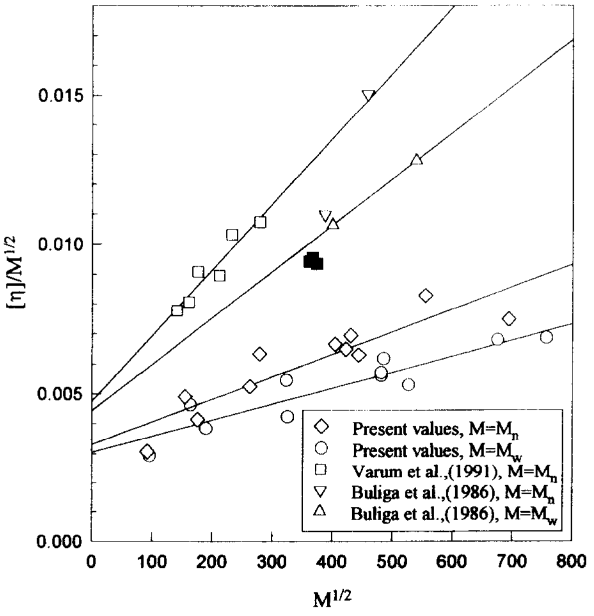

Fig. 5. The Stockmayer–Fixman plot to determine unperturbed dimensions, K_θ, for a random coil. Black symbols were not considered in the fitting.

From the K_θ value, the characteristic ratio (C_∞) can be obtained as:

$$C_\infty = (K_\theta/\Phi)^{2/3}(M_0)/b^2$$

M_0 is the molecular weight per residue ($M_0 = 162$) and b the distance between two consecutive glycosidic oxygens ($b = 0.532$ nm, value obtained by averaging over (1→4) and (1→3) links, with a 7/3 proportion between them).

Table 2 contains the values of C_∞ obtained from different K_θs. As it can be seen, C_∞ should be around 7, according to our results of unperturbed conditions. This is a comparatively low characteristic ratio, not typical of stiff chains. According to Buliga *et al.* (1986), C_∞ is around 18, a value that can be obtained from Fig. 5 if we equate K_θ to the single point having the highest $[\eta]$ $M^{-1/2}$ (the result reported by Buliga *et al.* (1986) for the highest M and using M_n). This implies no M dependence, hence the $[\eta]$ measured corresponds to θ conditions. As can be seen in Fig. 5 the overall picture is different, $[\eta]$ $M^{-1/2}$ varies with M, and the extrapolation is needed, instead of a single point value.

Theoretical calculations for the chain of β-glucan by Buliga *et al.* (1986) yield a result of $C_\infty = 11.7$, when considering the distribution of (1→3),(1→4) links as a second-order Markov process. It then appears that treating the β-glucan macromolecule as a coil, in which the molecular weight dependence of $[\eta]$ $M^{-1/2}$ is an excluded volume effect, tends to yield low values of C_∞, below theoretical predictions. The opposite point of view is to assume that such dependence is due solely to chain stiffness. The unperturbed dimensions of the coil are then to be obtained in the opposite limit of high Ms.

For stiff chains without excluded volume, the most adequate model is the worm-like cylinder. The parameters defining macromolecular dimensions are then the contour length of the chain, L, its Kuhn statistical segment length, l, or the persistence length, $p(=l/2)$, and the cross-section diameter or thickness of the molecule, d. According to that model, the intrinsic viscosity is given by:

$$[\eta] = \Phi(l/M)^{3/2} M^{1/2}(B_0 + A_0(l/L)^{1/2})^{-3}$$

with A_0 and B_0 functions of d/l. The parameters of the chain can be obtained from the plot proposed by Bohdanecký (1983): $(M^2/[\eta])^{1/3}$ vs $M^{1/2}$. This plot should be linear with slope, B_η, equal to B_0 $\Phi^{-1/3}$ $(M/Ll)^{1/2}$, and intercept, A_η, equal to A_0 $\Phi^{-1/3}$ M/L.

Figure 6 shows the Bohdanecký plot of our β-glucan samples, that keep a good linear behaviour. From the

Table 2. Values of the characteristic ratio, C_∞, obtained from different values of the unperturbed dimensions, K_θ (dl g$^{-3/2}$ mol$^{1/2}$), with the parameters of β-glucan

$K_\theta \times 10^3$	3	4	5	10	15
C_∞	6.3	7.6	8.9	14.4	18.4

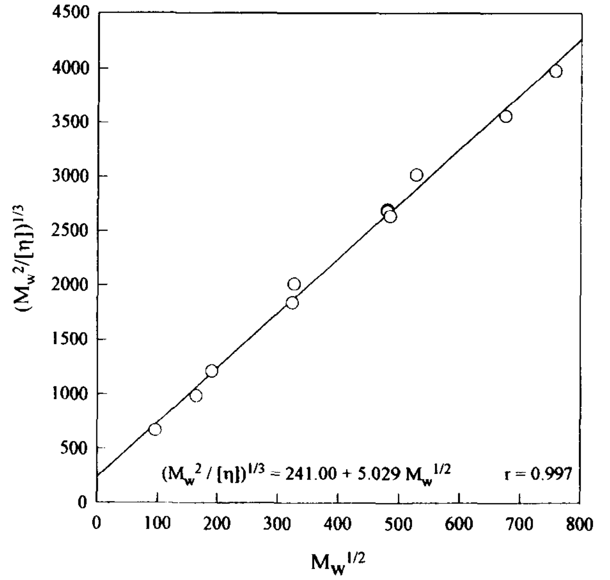

Fig. 6. The Bohdanecký plot of intrinsic viscosity according to the worm-like cylinder model.

fit, $A_\eta\ \Phi^{1/3} = 3.33\times10^9\,\mathrm{g\,mol^{-1}\,cm^{-1}}$, and $B_\eta\ \Phi^{1/3} = 6.92\times10^7\,\mathrm{g^{1/2}\,mol^{-1/2}\,cm^{-1}}$ were obtained. From these A_η and B_η values, the statistical segment length, l, or the persistence length, p, and the diameter, d, of the chain can be calculated if, M/L, A_0 and B_0 are known. As a first approximation for M/L, the value corresponding to a residue in the chain, namely equal to M_0/b, could be taken. The approximate equations proposed by Bohdanecký (1983) for A_0 and B_0, could be used:

$$A_0 = 0.46 - 0.53\log(d/l) \quad B_0 = 1.00 - 0.0367\log(d/l).$$

In this way, and always being within the range of validity of the approximate equations in this model (Bohdanecký, 1983), the results for l, p and d shown in Table 3 (first row) were obtained. The method used here to estimate M/L, takes M as mass per chain, which can be considered as a number average, but the plots of intrinsic viscosity are made with weight averages, M_w. The polydispersity of the β-glucan samples may then have some influence on the final results. We try to evaluate this influence by using M_n instead of M_w in the plots, namely by plotting $(M_n{}^2/[\eta])^{1/3}$ vs $M_n{}^{1/2}$. The results, thus obtained, are also shown in Table 3 (second row). We can see that they represent a slightly more stiff chain, but the differences are not important.

The figures shown in this table allow some interpretation about the stiffness of β-glucan chain. Values of statistical segment length 6.94–7.63 nm, mean that it spans 13–14 residues in the chain, which corresponds to an average of about four (1→3) links per statistical segment. It is usually accepted that the presence of (1→3) links is a source of flexibility in the otherwise rigid (1→4) sequences. Then, 13–14 residues containing an average of four (1→3) links are required to break the correlation between residues due to chain stiffness, and to form an uncorrelated statistical segment. Regarding the thickness of the molecule, the value $d = 0.45$ nm as the cross-section diameter is completely consistent with the geometry of the glucose residues. Thus, distances between atoms, substituting opposite positions in the ring, are only slightly above this value of d (for example, 0.48 nm between oxygens in C_2 and C_4), and the cross-section of the ring viewed laterally is smaller. Therefore, the value $d = 0.45$ nm is a very accurate average of the different cross-sections in the real chain.

Table 3. Persistence length, *p*, statistical segment length, *l*, cross-section diameter, *d*, and characteristic ratio, C_∞, of β-glucan, calculated assuming a worm-like cylinder model

	p(nm)	l(nm)	d(nm)	C_∞
M_w	3.47	6.94	0.45	13.0
M_n	3.81	7.63	0.45	14.3

The characteristic ratio, C_∞, can also be calculated from this worm-like description of the macromolecule. In the limit of high molecular weights, it is $(\langle R^2\rangle_0/M)_\infty = (B_0/(B_\eta{\cdot}\Phi^{1/3}))^2$. Then $C_\infty = \langle R^2\rangle_0/Nb^2$ can be obtained multiplying this $(\langle R^2\rangle_0/M)_\infty$ by M/Nb^2 or M_0/b^2. The result is entirely equivalent to l/b and it is shown in Table 3. This $C_\infty = 13.0$ corresponds to a more rigid chain than previously deduced from the coil treatment. It is also in better agreement with the theoretical calculations.

It can be concluded then, that according to the viscosity results, the chain of β-glucan is well modelled by a partially stiff worm-like cylinder, with rigidity characterised by the parameters shown in Table 3.

ACKNOWLEDGEMENTS

This work was supported by Cerveceros de España and the Comisión Interministerial de Ciencia y Tecnología (CICyT), Project ALI 93-0067.

REFERENCES

Bamforth, C.W. (1982). Barley β-glucans. Their role in malting and brewing. *Brew. Dig.*, **35**, 22–27.

Bohdanecký, M. (1983). New method for estimating the parameters of the wormlike chain model from the intrinsic viscosity of stiff-chain polymers. *Macromolecules*, **16**, 1483–1492.

Buliga, G.S., Brant, D.A. & Fincher, G.B. (1986). The sequence statistics and solution configuration of a barley (1→3),(1→4)-β-D-glucan. *Carbohydr. Res.*, **157**, 139–156.

Edney, M.J., Marchylo, B.A. & MacGregor, A.W. (1991). Structure of total barley β-glucan. *J. Inst. Brew.*, **97**, 39–44.

Fincher, G.B. (1975). Morphology and chemical composition of barley endosperm cell walls. *J. Inst. Brew.*, **81**, 116–122.

Forrest, I.S. & Wainwright, T. (1977). Differentiation between desirable and troublesome β-glucans. In *Eur. Brew. Conv., Proc. Congr. 16th*, Amsterdam, pp. 401–413.

Gómez, C., Navarro, A., Manzanares, P., Horta, A. & Carbonell, J.V. (1996). Physical and structural properties of barley (1→3),(1→4)-β-D-glucan. Part I. Determination of molecular weight and macromolecular radius by light scattering. *Carbohydr. Polym.*, **32**, 7–15.

Grimm, A., Krüger, E. & Burchard, W. (1995). Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer. *Carbohydr. Polym.*, **27**, 205–214.

Igarashi, O. & Sakurai, Y. (1966). Studies on the non-starchy polysaccharides of the endosperm of naked barley. *Agric. Biol. Chem.*, **30**, 642–645.

Izawa, M., Kano, Y. & Koshino, S. (1993). Relationship between structure and solubility of (1→3),(1→4)-β-D-glucan from barley. *J. Am. Soc. Brew. Chem.*, **51**, 123–127.

Kamide, K. & Saito, M. (1989). Viscometric determination of molecular weight. In *Determination of Molecular Weight. Chemical Analysis*, Vol. 103, eds A.R. Cooper & J.D. Winefordner. Wiley, New York.

Manzanares, P., Navarro, A., Sendra, J.M. & Carbonell, J.V. (1993). Determination of the average molecular weight of barley β-glucan within the range 30–100 kD by the calcofluor-FIA method. *J. Cereal Sci.*, **17**, 211–223.

McIntosh, G.H., Whyte, J., McArthur, R. & Nestel, P.J. (1991). Barley and wheat foods influence on plasma cholesterol concentrations in hypercholesterolemic men. *Am. J. Clin. Nutr.*, **53**, 1205–1209.

Navarro, A., Manzanares, P., Carbonell, J.V. & Sendra, J.M. (1995). Determination of (1→3),(1→4)-β-D-glucanase activity by a calcofluor-flow injection analysis method. *J. Cereal Sci.*, **22**, 275–284.

Sudaralingam, M. (1968). Some aspects of sterochemistry and hydrogen binding of carbohydrates related to polysaccharide conformations. *Biopolym.*, **6**, 189–213.

Vårum, K.M., Martinsen, A. & Smidsrød, O. (1991). Fractionation and viscometric characterisation of a (1→3),(1→4)-β-D-glucan from oat and universal calibration of a high-performance size exclusion chromatographic system by the use of fractionated β-glucans, alginates and pullulans. *Food Hydrocoll.*, **5**, 363–374.

Vårum, K.M., Smidsrød, O. & Brant, D.A. (1992). Light scattering reveals micelle-like aggregation in the (1→3),(1→4)-β-D-glucans from oat aleurone. *Food Hydrocoll.*, **5**, 497–511.

Wood, P.J., Weisz, J. & Blackwell, B.A. (1994). Structural studies of (1→3),(1→4)-β-D-glucans by ^{13}C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chem.*, **71**, 301–307.

Woodward, J.R., Fincher, G.B. & Stone, B.A. (1983). Water-soluble (1→3),(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydr. Polym.*, **3**, 207–225.