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# SOLID-STATE AND SOLUTION CONFORMATION OF SCLEROGLUCAN

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## ABSTRACT

Scleroglucan is a neutral polysaccharide composed of a linear chain of (1→3)-linked β-D-glucopyranosyl residues with (1→6)-linked β-D-glucopyranosyl groups attached to every third residue. The conformational behaviour of scleroglucan has been investigated in solution and in the solid state. Order–disorder transitions in aqueous solution were studied by measurement of intrinsic viscosity. The results indicate the occurrence of such a transition at a pH > 12, whereas gel formation under 10° is observed. X-Ray diffraction experiments performed on oriented fibers indicate that the backbone conformation is similar to that previously observed for curdlan, *i.e.*, a triple helix. The pendent (1→6)-linked β-D-glucopyranosyl residues protrude from the outside of the triplex, causing an expansion of the base plane parameters of the unit cell and further hampering lateral packing of the scleroglucan chains. The observed behaviour can be rationalized on the basis of a conformational analysis involving molecular modelling. As for the gentiobiose residue, extreme conformational flexibility about the (1→6)-β-linkage is disclosed. This conformational freedom is not significantly altered for the rotations about the (1→6)-β-linkage in the scleroglucan repeating-unit. Combination of solution and solid-state investigations provides insight into the aqueous gel-forming characteristics of scleroglucan.

## INTRODUCTION

Scleroglucan is the name given to a class of fungal polysaccharides secreted exocellularly by certain fungi of the genus *Sclerothinia*. The polysaccharide produced by *Sclerotium glucanicum*[1] has been widely studied, but it is the exopolymer from *Sclerotium rolfsii* that is produced commercially at this time. Scleroglucan is a neutral polysaccharide composed of a linear chain of (1→3)-linked β-D-glucopyranosyl residues with (1→6)-linked β-D-glucopyranosyl groups attached at about every third residue along the main chain.

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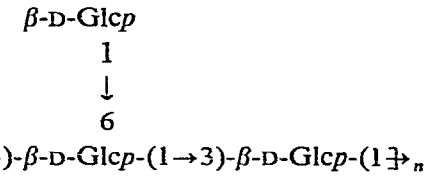
Its chemical structure was recently confirmed by n.m.r. measurements[2] as a regular polymer corresponding to a tetrasaccharide repeating-unit:




The optical rotary behaviour supports the concept (see below) that an ordered conformation exists in water and this is further corroborated by the absence of $^{13}C$-n.m.r. signals[2] for solutions in this solvent.

Polysaccharides secreted by different species of Sclerotium may differ somewhat in the number and length of side chains[3]. *S. glucanicum* has a single, (1→6)-linked β-D-glucopyranosyl group, whereas the commercial polysaccharide from *S. rolfsii* may have side chains of greater length. Scleroglucan from *S. glucanicum* has a d.p. of ~110, while the commercial scleroglucan used in this study has a d.p. of ~800 according to the commercial data available.

Refined grades of scleroglucan such as Biopolymer CS-11* dissolve readily in water, to give pseudoplastic solutions with good tolerance to a broad range of temperature, pH, and salt concentrations. Concentrations of 0.1–0.2% of scleroglucan in water can be used to stabilize 5–10% aqueous suspensions of such fine powders as zinc oxide. Scleroglucan has the caloric equivalent of starch in tests with rats[3]. Scleroglucan has possible uses as a replacement for many industrial gums. Potential exists in such areas as suspending, coating, and gelation in the food industry, and enhanced recovery of oil in the oil industry.

Due to the broad, potential, commercial value of scleroglucan, it became of interest to investigate the conformational parameters of this polysaccharide as it exists in the solid state. In particular, we wished to compare the solution and solid-state conformation of scleroglucan with that of curdlan, which contains a linear, (1→3)-linked chain of β-D-glucopyranosyl residues[4]. Curdlan exists in a triple-helical conformation[5] similar to that of (1→3)-β-D-xylan[6]. Also, extensive studies of curdlan in aqueous solutions of increasing pH have led to the hypothesis of an ordered to disordered transition[7].

## EXPERIMENTAL RESULTS

### *Solution properties*

The sample of scleroglucan used was provided by CECA, S.A. (Velizy, France) and is sold under the trade name Biopolymer CS*. Measurements of intrinsic viscosity in aqueous NaCl, using low-shear conditions in a Ubbelohde viscometer, led to the results shown in Fig. 1. The relationship between the reduced viscosity and pH shows a sharp transition at pH > 12 and, at the same time, a conformational transition can be demonstrated by optical rotation measurements[2]. This viscosity transition is not

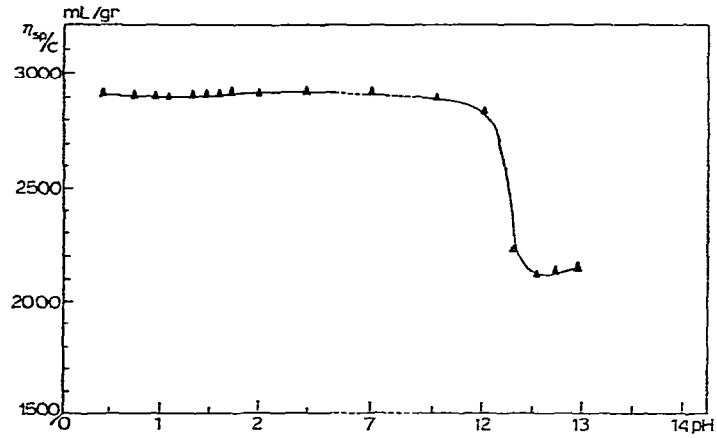



Fig. 1. Reduced viscosity ($\eta_{sp}/c$) of a solution of scleroglucan (0.49 g.L$^{-1}$) in NaCl ($0.8 \times 10^{-2}$M) as a function of the pH (pH decreased by HCl additions; pH increased by NaOH additions).

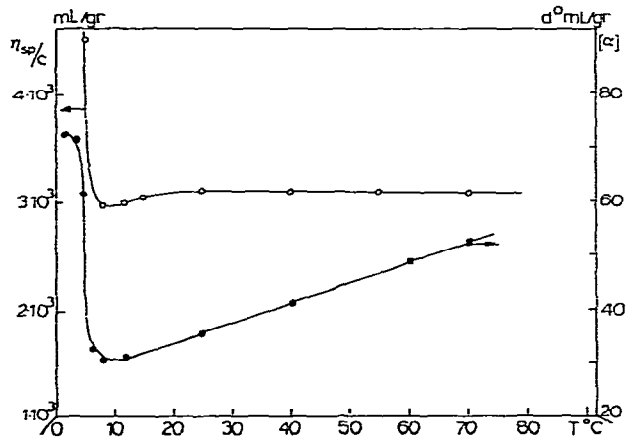



Fig. 2. Reduced viscosity ($\eta_{sp}/c$) and specific rotation ($[\alpha]_{300}$) of a solution of scleroglucan (0.72 g.L$^{-1}$) in water as a function of the temperature.

reversible due to a modification of molecular interactions and/or chemical modification, as deduced from $^{13}$C-n.m.r. measurements[2]. The results suggest that, in aqueous solution over a large range of pH (1–12), the polysaccharide adopts an ordered conformation associated with a large reduced viscosity. This situation could result from a complementary chain-interaction which enhances molecular size and chain stiffness by comparison with the random-coil conformation. The latter is assigned to the higher pH values, since the intrinsic viscosity at pH $>12$ is similar to that observed in dimethyl sulfoxide, a good solvent for (1→3)-β-D-glucans[8].

In Fig. 2, the reduced viscosity is given as a function of the temperature in dilute, aqueous solution. Below 10°, an increase of viscosity is associated with an increase of the optical rotary power. This behaviour corresponds also to the formation

of a gel at low temperature, as previously mentioned in the literature[9]. By contrast, the polysaccharide curdlan, which has the same backbone of (1→3)-linked β-D-glucosyl residues but lacks the D-glucosyl side-chains, forms a gel at 53° on heating a powder suspension. This reflects the need for heat to overcome the strong, inter-chain, association forces inside each particle of curdlan. In scleroglucan, gel formation is probably due to more weakly interacting, triple-helix segment entwinements (see below).

*X-Ray diffraction*

Oriented fibers were prepared in a manner similar to the preparation of curdlan fibers[10]. A 15% solution of scleroglucan in dimethyl sulfoxide was extruded into a methanol bath at room temperature. After washing in a water bath, the fibers were allowed to dry at constant length. The "as spun" fibers were then annealed under tension in a sealed bomb at 145° in an atmosphere of 100% relative humidity.

X-Ray diagrams of the "as spun" (Fig. 3) and "annealed" (Fig. 4) fibers were

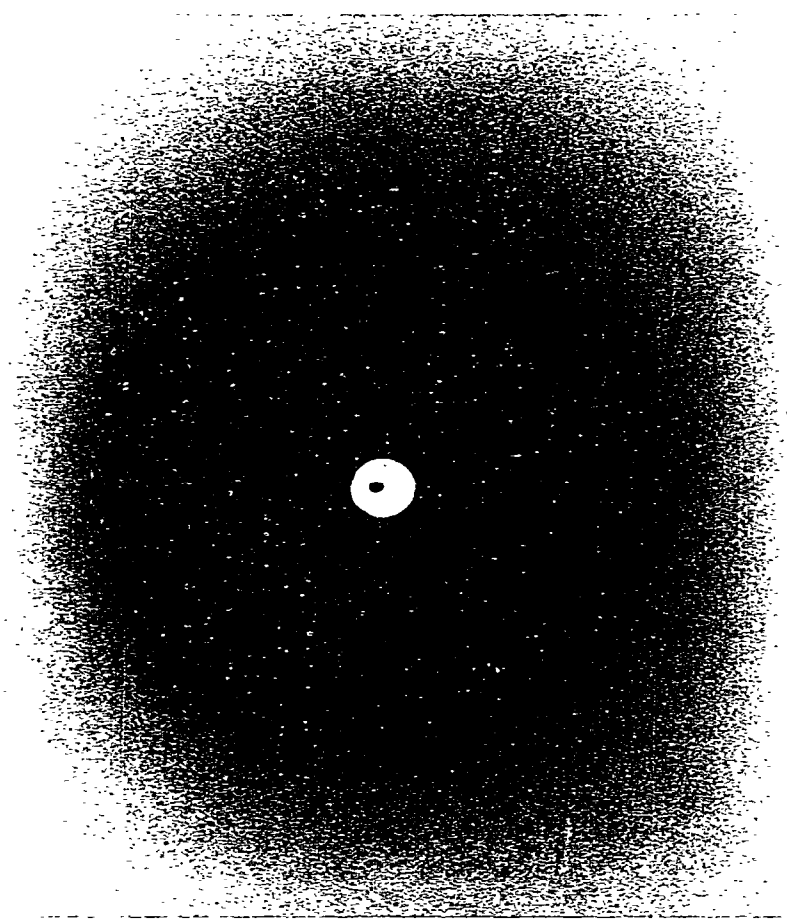


Fig. 3a

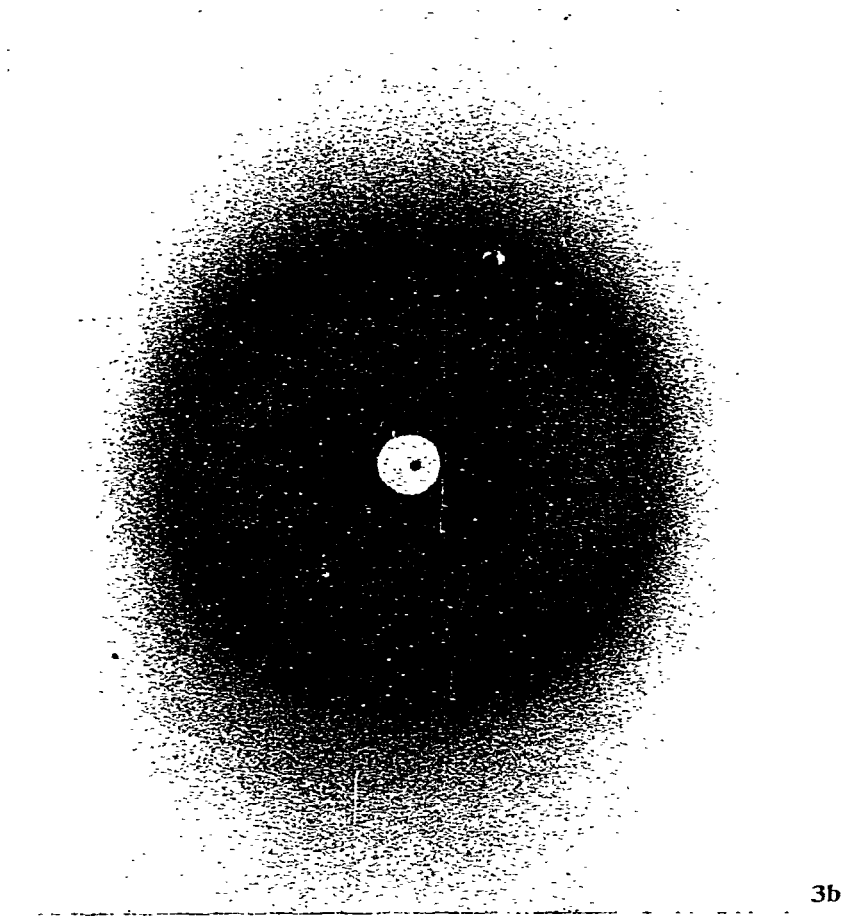



Fig. 3. X-Ray fiber diagrams of a, curdlan "as spun"; and b, scleroglucan "as spun".

recorded in a Warhus flat-film camera both in air at ambient condition and under vacuum. CuK$\alpha$ radiation ($\lambda$ = 1.5418 Å) and Kodak no screen X-ray film were used. The unit-cell parameters were estimated from measurement of the *d*-spacings of five reflections, using NaF powder as calibration.

During extrusion of scleroglucan fibers, it was noted that they had less strength than similarly produced curdlan fibers and a corresponding, higher degree of swelling. The fibers dried at constant length by wrapping around a glass cylinder had an ellipsoidal cross-section.

Measurement of *d*-spacings on the fiber diagrams obtained from scleroglucan indicates that the unit-cell dimensions do not change during annealing or the subsequent drying and humidifying experiments (see Table I). This observation is at variance with those for hydrous and anhydrous polymorphs of curdlan (see Table II). However, the unit cell of scleroglucan is hexagonal, as is that of curdlan, with a fiber

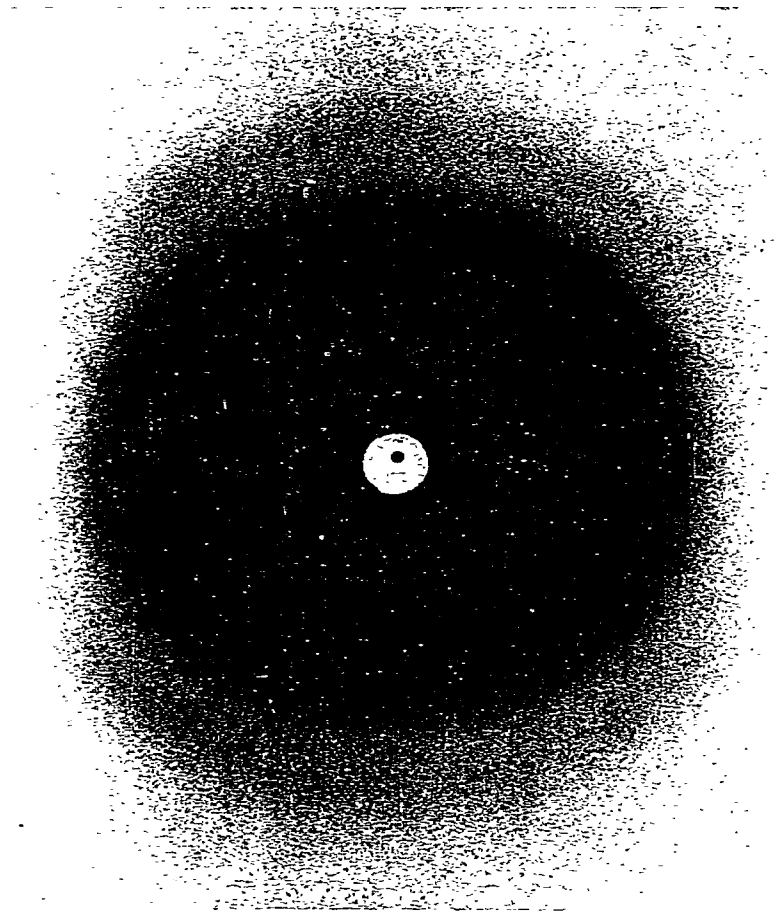


Fig. 4. X-Ray fiber diagram of scleroglucan annealed at 45°.

TABLE I

OBSERVED BRAGG SPACINGS

| *Curdlan* | | | | *Scleroglucan* | | | |
|---|---|---|---|---|---|---|---|
| *As spun* | | *Annealed (145°)* | | *As spun* | | *Annealed (145°)* | |
| d *(Å)* | *(hkl)* | d *(Å)* | *(hkl)* | d *(Å)* | *(hkl)* | d *(Å)* | *(hkl)* |
| 14.5 | (100) | 12.6 | (100) | 14.9 | (100) | 15.0 | (100) |
| 8.3 | (110) | 7.3 | (110) | — | — | 7.5 | (200) |
| 7.0 | (200) | 6.3 | (200) | 4.2 | (211) | 4.5 | (201) |
| 4.5 | (203) | 4.5 | (111) | | | | |

TABLE II

HEXAGONAL UNIT-CELLS

| *As spun* | *Annealed (145°) (dry)* | *Annealed (145°) (wet)* |
|---|---|---|
| *Curdlan* | | |
| $a = b = 16.8$ Å | $a = b = 14.3$ Å | $a = b = 15.6$ Å |
| $c = 22.2$ Å | $c = 5.9$ Å | $c = 18.8$ Å |
| *Scleroglucan* | | |
| $a = b = 17.3$ Å | $a = b = 17.3$ Å | $a = b = 17.3$ Å |
| $c = 6.0$ Å | $c = 6.0$ Å | $c = 6.0$ Å |

repeat very near that of anhydrous curdlan. In fact, there is a great similarity between the fiber diagrams of curdlan and scleroglucan (see Fig. 3).

Unit-cell parameters for scleroglucan are $a = b = 17.3$ Å, $c$ (fiber repeat) = 6.0 Å, and $\gamma = 120°$, expanded somewhat when compared with the unit-cell parameters of dry curdlan: $a = b = 14.3$ Å, $c$ (fiber repeat) = 5.9 Å, and $\gamma = 120°$. The density[6] of dry curdlan fibers is 1.49 g.cm$^{-3}$, while that of dry scleroglucan fibers is 1.43 g.cm$^{-3}$, as measured by flotation in a mixture of toluene–chloroform.

The similarity between the unit cells of curdlan and scleroglucan suggests that the structure of scleroglucan is a triple helix (a triplex), as is the curdlan structure[5]. As in curdlan, the scleroglucan triplex consists of three, symmetry-related, individual strands composed of six residues in the backbone per turn. The unit-cell parameters of scleroglucan along with the density indicates that approximately eight D-glucopyranosyl residues are found in the unit cell. Due to symmetry considerations, the unit cell contains only one third of the triplex. The hydroxymethyl groups of curdlan protrude from the outside of the triple helix, and the three strands of the triplex are held together by interstrand hydrogen-bonds. Therefore, it is conceivable that the (1→6)-linked β-D-glucopyranosyl side-groups of scleroglucan could be accommodated without disturbing the triple helix. A similar effect is observed when comparing the structures of (1→3)-β-D-xylan[6] and curdlan[5], where a hydroxymethyl group is present at the exterior of the curdlan triplex and not present in the (1→3)-β-D-xylan.

*Conformational analysis of gentiobiose and scleroglucan repeating-unit*

The relative orientation of two contiguous, (1→6)-linked β-D-glucosyl residues is described by the three torsion angles: $\Phi$, $\psi$, and $\omega$, as depicted in Fig. 5 where: $\Phi$ = H-1–C-1–O-1–C-6′, $\psi$ = C-1–O-1–C-6′–C-5′, and $\omega$ = O-1–C-6′–C-5′–H-5′. In order to avoid confusion in defining one of the methylene hydrogen atoms as reference, the angle $\psi$ is referred to C-5′ rather than to a hydrogen atom on C-6′. Another angular parameter to be used is $\omega$ (O-5′), the same torsional angle as the defined angle $\omega$. For the ideal tetrahedral arrangement, these angles differ by 120°.

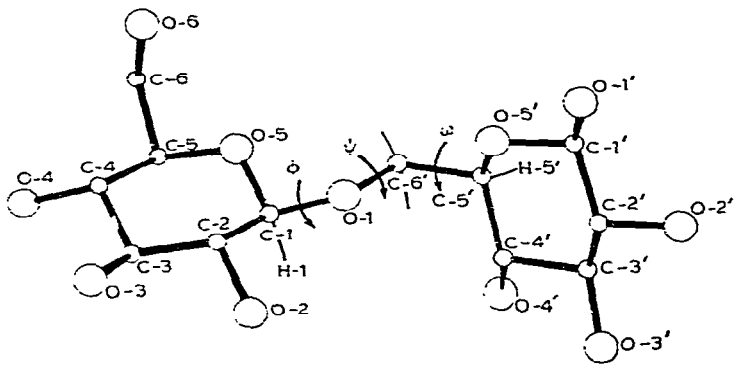



Fig. 5. View of the molecule of gentiobiose as found in the solid state, along with the numbering of the atoms and the pertinent torsion-angles (PITMOS, 1980)[22].

The sign of the dihedral angle is defined according to the rules recommended by the IUPAC–IUB Commission of Biochemical Nomenclature[11].

The geometry used for the gentiobiose unit was that determined from X-ray crystallography[12]. The valence angle ($\tau$) at the glycosidic oxygen atom was kept at 113.3°. The potential energy was computed by taking into account the van der Waals, torsional, and hydrogen-bond contributions; the van der Waals interactions between non-bonded atoms were evaluated with the parameters proposed by Scott and Scheraga[13,14]. A three-fold, intrinsic, torsional potential was used for rotations around the C-1–O-1, O-1–C-6′ and C-6′–C-5′ bonds with barriers of 0.9, 2.7, and 3.0 kcal/mol, respectively[15,16]. Furthermore, a maximum anomeric stabilization of 1.0 kcal/mol was associated with the $\Phi$ rotation[17] and an energy of 3 kcal/mol was associated with the $\omega$ rotation for the value $\omega = 60°$, in order to account for an instability arising from stereoelectronic interactions for this given conformation[18]. Hydrogen-bond energies were calculated by an empirical[19], inverse, third-power expression $V_{hb} = -55.0/R^3$, where R is the distance between oxygen atoms, which should lie between 2.5 and 3.1 Å. The total potential-energy for different values of $\Phi$, $\psi$, and $\omega$ was calculated by using the same fundamental approach as for the two-dimensional ($\Phi$,$\psi$) maps, except that a series of maps was constructed, each one corresponding to a different value of $\omega$. These calculations were made at 30° intervals in the value of $\omega$, over the range 0–360°. Each two-dimensional ($\Phi$,$\psi$) map was constructed for the full, angular range over 5° intervals. The same strategy was used for the conformational analysis of the scleroglucan repeating-unit. The calculations were performed on a Honeywell-Bull CII "HB68" computer at the Université de Grenoble.

Conformations about the (1→6)-linkage were plotted in terms of iso-energy contours covering the range 0–10 kcal/mol of relative energies. The results may be represented by a three-dimensional drawing with $\Phi$, $\psi$, and $\omega$ as variables. The calculations indicate that the asymmetric volume is best described as a cylinder having

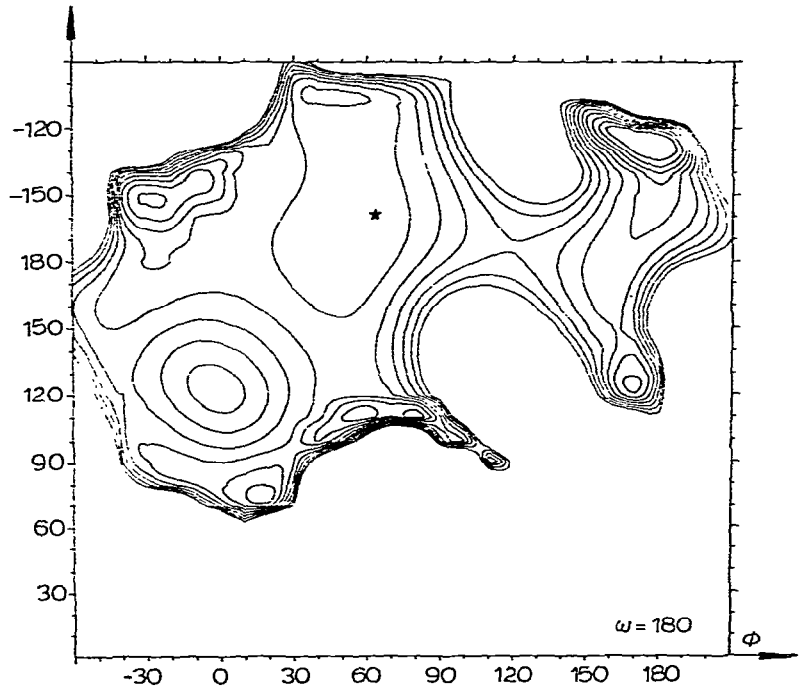



Fig. 6. A ($\Phi$, $\Psi$) section having $\omega = 180°$, for (1→6)-linked β-D-glucosyl residues. Relative isoenergy contours are drawn at intervals of 1 kcal/mol; * indicates the crystallographic conformation observed for gentiobiose.

TABLE III

FAVORED CONFORMATIONS FOR GENTIOBIOSE, FOR THE SELECTED VALUES OF $\omega$

| $\omega$ *(degrees)* | $\Phi$ *(degrees)* | $\Psi$ *(degrees)* | $\Delta E$ *(kcal/mol)* |
|---|---|---|---|
| 180 | 180 | −130 | 0.0 |
| (*gauche–gauche*; $\omega^{O-5} = -60°$) | −30 | −150 | 1.96 |
| | 60 | 110 | 2.19 |
| | 80 | 110 | 2.19 |
| | 170 | 130 | 4.06 |
| | −110 | 50 | 4.10 |
| | 20 | 80 | 4.13 |
| | 50 | −20 | 4.17 |
| −60 | 20 | −90 | 2.33 |
| (*gauche–trans*; $\omega^{O-5} = 60°$) | −30 | −140 | 2.92 |
| | 30 | 60 | 3.46 |
| | 50 | −10 | 4.42 |
| | 170 | −90 | 4.73 |
| 60 | 50 | −70 | 3.99 |
| (*trans–gauche*; $\omega^{O-5} = 180°$) | 60 | 120 | 5.21 |
| | 50 | 130 | 5.42 |

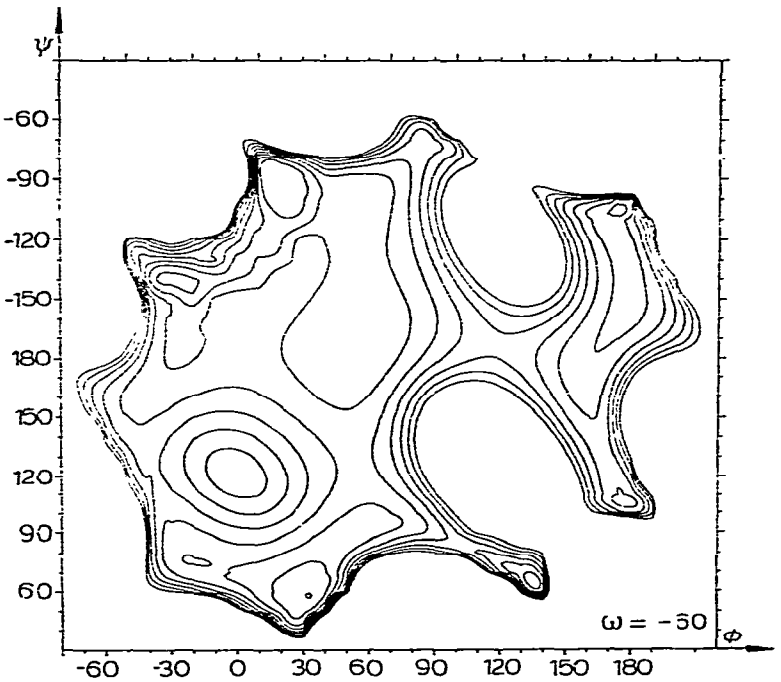



Fig. 7. Two-dimensional, iso-energy map: ($\Phi$, $\Psi$) section having $\omega = -60°$ for (1→6)-linked $\beta$-D-glucosyl residues.

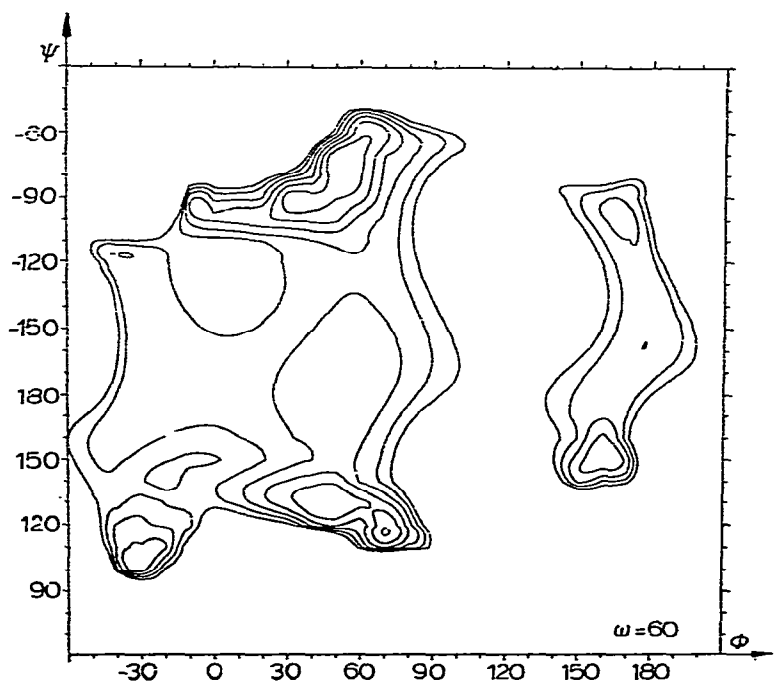



Fig. 8. Two-dimensional, iso-energy map: ($\Phi$, $\Psi$) section having $\omega = 60°$ for (1→6)-linked $\beta$-D-glucosyl residues.

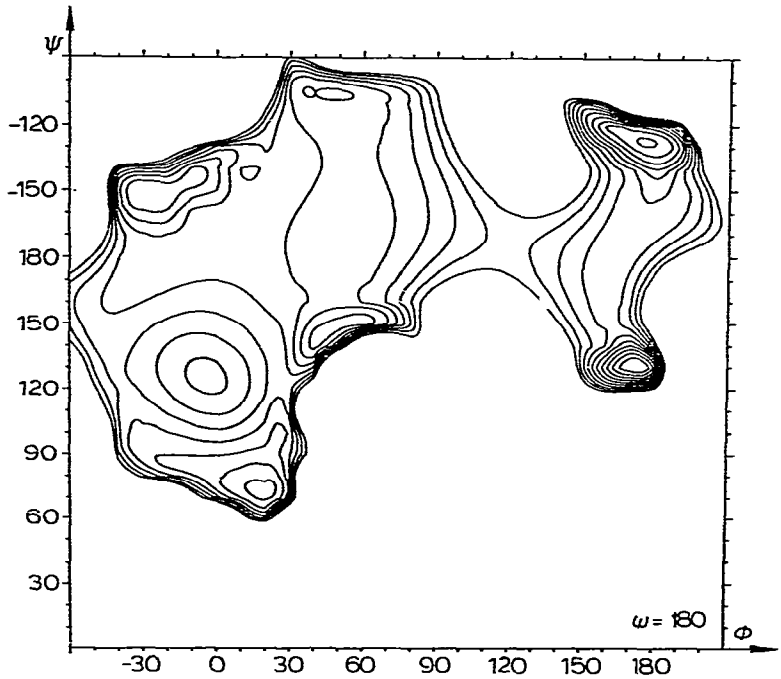



Fig. 9. Two-dimensional, iso-energy map: ($\Phi$, $\Psi$) section having $\omega = 180°$ for (1→6)-linked $\beta$-D-glucosyl residues in scleroglucan repeating-unit. Relative iso-energy contours are drawn at intervals of 1 kcal/mol.

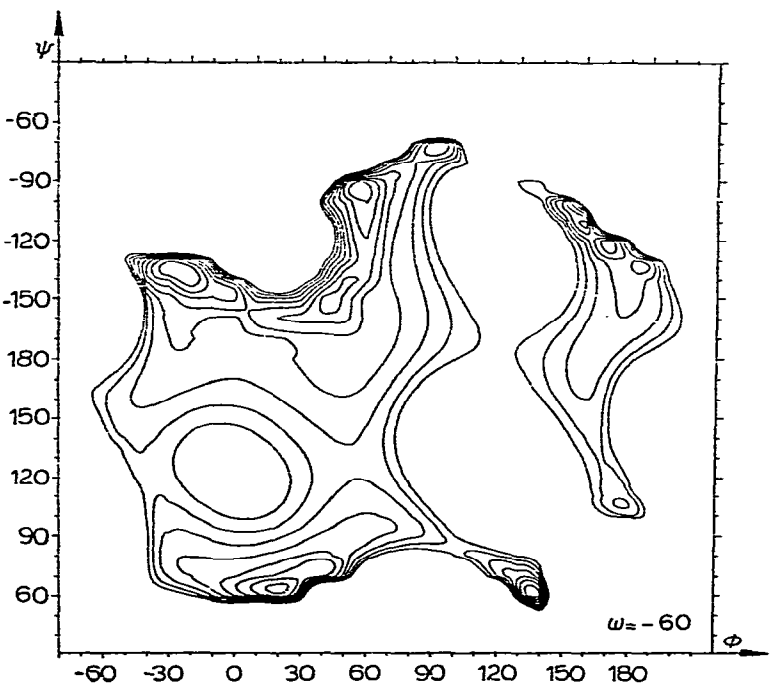



Fig. 10. Two-dimensional, iso-energy map: ($\Phi$, $\Psi$) section having $\omega = -60°$ for (1→6)-linked $\beta$-D-glucosyl residues in scleroglucan repeating-unit.

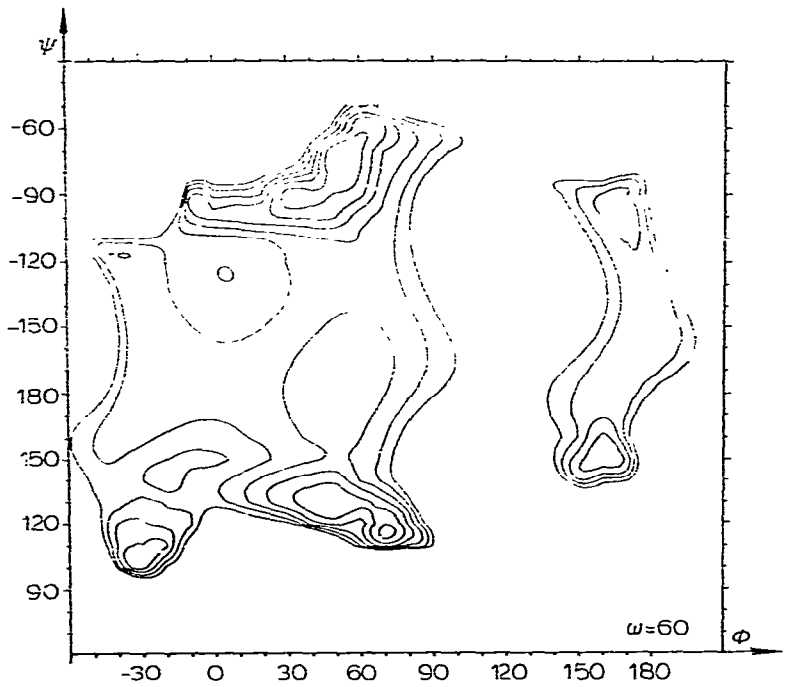



Fig. 11. Two-dimensional, iso-energy map: ($\Phi$, $\Psi$) section having $\omega = 60°$ for (1→6)-linked β-D-glucosyl residues in scleroglucan repeating-unit.

three favoured levels of $\omega$, corresponding to the non-eclipsing conformations $\omega = -60°$, 180°, and 60°. For these values of $\omega$, in the energy range assumed, the ($\Phi$, $\psi$) iso-energy contours are shown in Figs. 6–8. However, it should be noted that the energy differences occurring between levels of $\omega$ over the range 0–360° arise essentially from the intrinsic torsional potential for the rotation about the C-6′–C-5′ bond, and that no drastic steric interactions are observed. This behaviour is quite different from that found for the (1→6)-α-linked disaccharide[20], where the levels of $\omega$ could be discriminated on the basis of the conjunction of different contributors to the calculated energy. Therefore, none of the selected levels of $\omega$ shown in the present work can be readily discarded. For the selected, non-eclipsing conformations of $\omega$, the energy minima along with the associated energy values, are given in Table III. These values are in agreement with those reported previously[21]. As expected, extreme conformational flexibility is disclosed by the calculations. The $\Phi$ values are distributed over a large range, the lowest energy values are found in the interval −60 to 100°, but conformations centered about $\Phi = 180°$ are also found; $\Psi$ values are distributed over more than 180° (−60 to 60°). The crystalline conformation of gentiobiose[12] ($\Phi = 63.2°$, $\psi = -156.3°$, $\omega^{O-5} = -61.5°$), which is ~4 kcal/mol above the energy minimum, is found in the region shown in Fig. 6.

The scleroglucan repeating-unit was generated with $\Phi$ and $\psi$ angles about the (1→3)-β-linkage having values corresponding to the triple-helix structure of curdlan

($\Phi = 29.1°, \psi = 9.6°$). These values were kept constant during the calculations, and only the conformations about the (1→6)-β-linkage were investigated. Here again, the conformations were plotted in terms of iso-energy contours covering the range 0–10 kcal/mol of relative energy. For the three values of $\omega$, corresponding to the non-eclipsing conformations, the ($\Phi$, $\psi$) iso-energy contours are shown in Figs. 9–11. Comparison of the iso-energy contours with these obtained for gentiobiose discloses that the enormous conformational freedom is not significantly altered for rotations about the (1→6)-β-linkage in the scleroglucan repeating-unit. Corresponding maps calculated for rotation $\omega = -60°$, about the C-5′–C-6′ bond, are identical. This $\omega$ conformation corresponds to a spatial arrangement about the (1→6)-β-linkage such that the gentiobiose unit is "folded" onto itself. This explains why interactions further away than the first neighbours are not significant in this case. As for the two other conformations about $\omega$, introduction of the (1→3)-β-linked trisaccharide, instead of a single D-glucosyl group, only brings minor restrictions in the space of allowed conformations about the (1→6)-β-linkage.

From the present calculations, it may be deduced that the pendent (1→6)-linked β-D-glucosyl groups found in scleroglucan will have little effect on the architectural features of the backbone made up of (1→3)-linked β-D-glucan chains, *i.e.*, the triple-helical arrangement. Conversely, the (1→6)-β-linked substituents along the chain are likely to exhibit extreme conformational flexibility. In terms of solid-state arrangement, such flexibility will encourage disorder of the lateral glucose residues, thereby hampering lateral packing of the scleroglucan chains.

## DISCUSSION

While the aqueous gel-forming characteristics of scleroglucan are not as marked as for curdlan, evidence is accumulating that a complementary interaction of the chains producing pseudo-crosslinks in solution is responsible for gelation and/or ordering. In the experiments to produce fibers for the X-ray diffraction study, the gel-swelling of the "as spun" scleroglucan fibers in methanol was at least twice that observed for curdlan. Similarly in water, solution rather than gelation is the rule. Clearly, the presence of the (1→6)-β-D-glucosyl substituent has added a measure of steric stabilization to individual chains and to the surface of triple-helical segments compared to curdlan. This additional drive towards solution in water is responsible for the difference in observed behaviour between these two similar polysaccharides.

Model building using the molecular parameters derived for curdlan shows that the same triple-helix organization can be achieved with both polysaccharides. In scleroglucan, where the X-ray data show that a larger base plane unit-cell is present, the triple helices are coated with the (1→6)-β-D-glucosyl substituents. Because of their intrinsic mobility, these groups will be mobile even in the solid state, especially when hydration is extensive. In the latter case, one can think of the triple helices as forming a paracrystalline state, because translation/rotation of parallel triple-helix segments are seldom in crystallographic register.

Such cell-wall and seed polysaccharides as 4-*O*-Me-glucuronoxylan, arabinoxylan, and galactomannans also achieve solubility due to single, substituent carbohydrate units. However, these substituents are not regularly spaced and solubility is aided by the fact that they hinder formation of the most stable crystal lattice. For scleroglucan, the substituent occurs regularly, and hence there is a potential for full, three-dimensional ordering of all the chain components. The problem is the numerous, rotational, isomeric states of the (1→6)-β-D-glucosyl groups, at least under the conditions so far investigated.

In this context, the conclusion that native *Schizophyllum commune* can be dispersed in water as a rigid-rod, triple helix by ultrasonification is interesting[8]. It suggests that the biosynthesis involves simultaneous polymerization and crystallization, so that the co-operative cohesion of the triple-helix segments is maintained even on dispersion in water.

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REFERENCES

1 M. E. SLODKI AND L. J. WICKERHAM, *J. Gen. Microbiol.*, 42 (1966) 381–385.
2 M. RINAUDO AND M. VINCENDON, *Biopolymers*, (submitted).
3 P. A. SANDFORD, *Adv. Carbohydr. Chem. Biochem.*, 36 (1979) 265–313.
4 T. HARADA, K. MASADA, K. FUJIMORI, AND I. MAEDA, *Agric. Biol. Chem.*, 30 (1966) 196–198.
5 Y. DESLANDES, R. H. MARCHESSAULT, AND A. SARKO, *Macromolecules*, 13 (1980) 1466–1471.
6 E. D. T. ATKINS AND K. D. PARKER, *J. Polym. Sci., Part C*, 28 (1969) 69–81.
7 K. OGAWA, T. WATANABE, J. TSURUGI, AND S. ONO, *Carbohydr. Res.*, 23 (1972) 399–405.
8 T. YANAKI, T. NORISUYE, AND H. FUJITA, *Macromolecules*, 13 (1980) 1462–1466.
9 N. E. RODGERS, in R. L. WHISTLER (Ed.), *Industrial Gums*, Academic Press, New York, 1973, ch. 22.
10 R. H. MARCHESSAULT, Y. DESLANDES, Y. K. OGAWA, AND P. R. SUNDARARAJAN, *Can. J. Chem.*, 55 (1977) 300–303.
11 IUPAC–IUB Commission on Biochemical Nomenclature, *Arch. Biochem. Biophys.*, 145 (1971) 405–621; *J. Mol. Biol.*, 52 (1970) 1–17.
12 F. ARENE, A. NEUMAN, AND F. LONGCHAMBON, *C. R. Acad. Sci., Ser. C*, 288 (1979) 331–334; D. C. ROHRER, A. SARKO, T. L. BLUHM, AND Y. N. LEE, *Acta Crystallogr., Sect. B*, 36 (1980) 650–654.
13 R. A. SCOTT AND H. A. SCHERAGA, *J. Chem. Phys.*, 44 (1966) 3054–3069.
14 R. A. SCOTT AND H. A. SCHERAGA, *J. Chem. Phys.*, 45 (1966) 2091–2101.
15 I. TVAROSKA AND T. BLEHA, *Tetrahedron Lett.*, (1975) 249–252.
16 I. TVAROSKA AND T. BLEHA, *J. Mol. Struct.*, 24 (1975) 249–259.
17 S. PÉREZ, Thèse de Doctorat, Université Scientifique et Médicale de Grenoble, 1979.
18 R. H. MARCHESSAULT AND S. PÉREZ, *Biopolymers*, 18 (1979) 2369–2374.
19 J. BLACKWELL, A. SARKO, AND R. H. MARCHESSAULT, *J. Mol. Biol.*, 42 (1969) 379–383.
20 I. TVAROSKA, S. PÉREZ, AND R. H. MARCHESSAULT, *Carbohydr. Res.*, 61 (1978) 97–106.
21 S. MELBERG AND K. RASMUSSEN, *Carbohydr. Res.*, 78 (1980) 215–224.
22 M. L. DHEU AND S. PÉREZ, *1980 PITMOS*, Programmes Interactifs de Tracés de Molécules et de Structures, C.E.R.M.A.V. (CNRS), Grenoble, France.