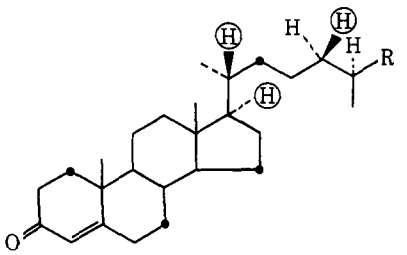
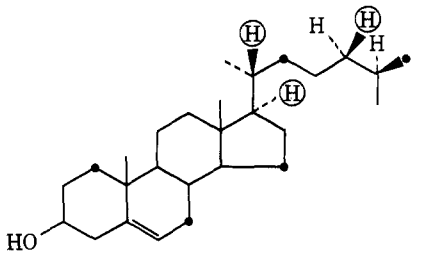


19a, R = $\overset{\bullet}{C}HO$
b, R = H



20

Walborsky and Allen[34] on the mechanism of decarbonylation of aldehydes with tris(triphenylphosphine)-rhodium chloride confirm our observations.

(34) H. M. Walborsky and L. E. Allen, *J. Amer. Chem. Soc.*, **93**, 5465 (1971).

In any event, it is abundantly clear that the terminal carbon of cholesterol, derived from C-2 of mevalonic acid, bears the oxygen function. Since the 26-hydroxycholestenone has the 25*S* configuration as in **18**a, it follows that cholesterol must have configuration **20**. This taken together with the proven addition of a 24-*pro-S* hydrogen indicates that reduction of the $\Delta^{24}$ of lanosterol by the enzymes of the S-10 fraction of rat livers can be formally considered as a cis addition of two hydrogens. In contrast the reduction of $\Delta^{24}$ of the sterol precursor of tigogenin in *Digitalis lanata* was recently shown to be equivalent to a trans reduction involving the addition of a 24-*pro-R*-hydrogen.[35]

**Acknowledgment.** This work was supported by grants from the National Institutes of Health, AM12156, HE10566, CA-K3-16614, and the National Science Foundation, GB8277. We are greatly indebted to Professor Kurt Schubert of the Zentral Instut für Mikrobiologie und Experimentelle Therapie of the Deutsche Akademie der Wissenschaften zu Berlin in Jena (DDR) for the specimen of *M. smegmatis*. Thanks are due to Professor Ch. Tamm of the University of Basel and Professor D. Arigoni of the E. T. H. in Zurich for letting us know their results prior to their publication.

(35) L. Canonica, F. Ronchetti, and G. Russo, *J. Chem. Soc., Chem. Commun.*, 1309 (1972).

# Crystalline Chain Conformation of Mycodextran[1a]

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**Abstract:** Mycodextran, also known as nigeran, is a linear polysaccharide consisting of α-D-glucose units joined by alternating α-1→4′ and α-1→3′ linkages. X-Ray analysis of polymer single crystals of mycodextran with the folded-chain structure led to an orthorhombic unit cell of dimensions $a$ = 17.6 Å, $b$ = 6.85 Å, and $c$ (fiber axis) = 13.4 Å, with a twofold screw axis along the chain, for the crystalline polymer. A conformational analysis method based on minimum energy considerations and allowing four rotatable bonds shows that a hydrogen bond is possible between the O-2 and O-3′ hydroxyls of contiguous residues in the α-1→4′ linkage and another between the O-2 O-4′ hydroxyls of the residues in the α-1→3′ linkage. The results obtained by means of two different approaches, namely the one which analyzes conformations in terms of the angles $\phi$ and $\psi$ and the other which considers the rotation of the residues around a virtual bond, are compared. The crystalline conformation of mycodextran is shown to be a "corrugated ribbon." An energetically favored scheme of chain folding in mycodextran is proposed.

Mycodextran, also known as nigeran, is a naturally occurring fungal polysaccharide. It was first isolated by Dox and Niedig[2] from the species *Penicillium expansum* and *Aspergillus niger*. More recently, Reese and Mandels[3] found that mycodextran constitutes up to 40% of the dry weight of the mycelium of some species of *Aspergillus* and *Penicillium* genera. Tung and Nordin[4] have shown that mycodextran is a major constituent of the mycelium cell wall of *Aspergillus niger*. Reese and Mandels[3] also isolated an α-D-glucanase having high specificity for mycodextran.

Chemical investigations[5-7] have shown that mycodextran is an exclusively α-D-glucan, composed of units linked together alternately through 1→3′ and 1→4′ linkages, as shown in Figure 1. The polymer may be considered either as a polymaltose with α-1→3′ linkages or as a polynigerose with α-1→4′ linkages. The repeating unit of this polysaccharide is therefore

(1) (a) Taken in part from the Ph.D. Thesis by G. J. Quigley, College of Forestry, 1969; (b) Université de Montréal; (c) College of Forestry.

(2) (a) A. W. Dox and R. E. Niedig, *J. Biol. Chem.*, **18**, 167 (1914); (b) A. W. Dox, *ibid.*, **20**, 83 (1915).

(3) E. T. Reese and M. Mandels, *Can. J. Microbiol.*, **10**, 103 (1964).

(4) K. H. Tung and J. H. Nordin, *Biochem. Biophys. Res. Commun.*, **28**, 519 (1967).

(5) S. A. Barker, E. J. Bourne, and M. A. Stacey, *J. Chem. Soc.*, 3084 (1953).

(6) S. A. Barker and T. R. Carrington, *ibid.*, 3588 (1953).

(7) S. A. Barker, E. J. Bourne, D. M. O'Mant, and M. A. Stacey, *ibid.*, 2448 (1957).

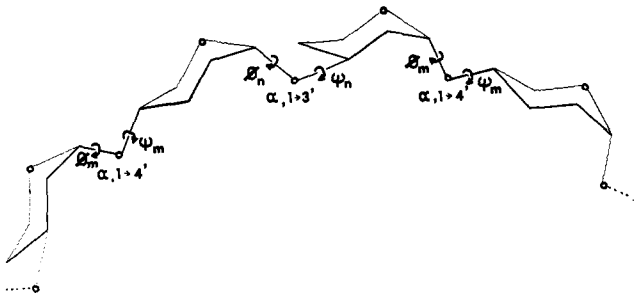



Figure 1. Schematic representation of the mycodextran chain, with alternate $\alpha$-1→4′ and $\alpha$-1→3′ linkages. The conformational angles ($\phi_m$, $\psi_m$) and ($\phi_n$, $\psi_n$) are shown.

a disaccharide. Mycodextran, though limited to a particular species in occurrence and having little commercial importance, compared with its close relative amylose, is of prime interest to enzymologists.

By combining X-ray fiber diffraction data with stereochemical analysis of the macromolecular chain, Rees and coworkers[8,9] have succeeded in the structural analysis of a regular copolysaccharide. Since many immunochemically important polysaccharides are regular copolymers, this approach augurs well for understanding the properties of these substances as well as extending the scope of conformational analysis methods for polysaccharides. In this study of the crystalline conformation of mycodextran, X-ray diffraction, electron microscopy, and stereochemical methods have been used.

## Experimental Section

Several samples of mycodextran were kindly provided by Dr. E. T. Reese, U. S. Army Quartermaster Corps, Natick, Mass. The samples had all the notable features of the infrared spectrum reported for this polysaccharide.[10] The measured intrinsic viscosity of 0.278 dl/g of the triacetate derivative corresponds to a degree of polymerization, determined in chloroform *via* a Mechrolab vapor phase osmometer, of 20. Such a low degree of polymerization renders all attempts to prepare oriented films or fibers ineffective, as will be described subsequently.

Mycodextran is soluble only in hot water, usually boiling. If a concentrated solution of mycodextran in hot water is spread on a Teflon plate and allowed to cool, a thick white paste forms, which on drying breaks into small hard chunks which are extremely brittle. In a mixture of DMSO and water at 90°, mycodextran forms a surface skin, which can be removed with care. Though the films obtained in this manner were flexible, all attempts to stretch them were unsuccessful.

Folded chain lamellar single crystals of mycodextran were prepared as follows: a 0.1% solution of the sample dissolved in boiling water was subsequently placed in a constant-temperature bath. The crystals were allowed to grow for nearly 48 hr. After this period of time, the solution appears quite turbid. The suspension was then rapidly cooled to room temperature and the crystals were collected by centrifugation. The optimum conditions for the growth of the crystals were found to be at a concentration of 0.01 g of mycodextran per 100 ml of water and a growth temperature of 60°.

To obtain oriented X-ray fiber diagrams from polymer single crystals, it is necessary to stack many of them together. When a paste of the single crystals has been spread onto a plate, the surface forces that develop during the drying process tend to make the large faces of the crystals align parallel to the plate. Assuming that the polymer chains run approximately normal to the platelet faces, as is frequently observed,[11] the fiber direction will be in the direction which is perpendicular to the mat of the single crystals. It is therefore possible to obtain a fiber diagram by directing the X-ray beam into the edge of the mats, parallel to the widest crystal faces. A schematic of the resulting fiber diagram for mycodextran is shown in Figure 2.

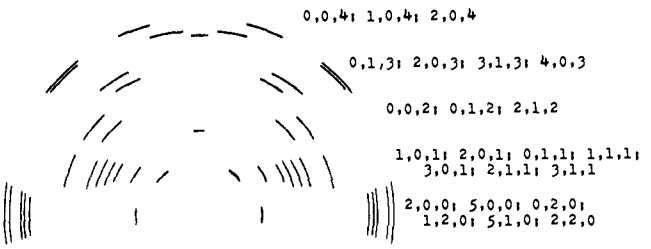



Figure 2. A schematic of the X-ray pattern, obtained from a stack of single-crystal mats of mycodextran. The apparent layer lines are marked.

The quality of the actual fiber diagram left much to be desired in terms of layer-line separation as defined by the perfection of the chain axis orientation. In addition, there was diffuse scattering in the central part of the diagram which is probably related to the powder character of the sample. Finally, the crystalline structure was found to be somewhat dependent on humidity; under conditions of high temperature (110°) and vacuum, noticeable changes in the diffraction pattern occurred. This work deals only with that form of mycodextran which occurs under normal laboratory conditions of temperature and humidity. Because of the quality of the X-ray patterns and other shortcomings (*cf.* above), quantitative intensity measurements were not attempted.

Electron micrographs were obtained by diluting the single crystal suspension with distilled water to an appropriate concentration and placing a drop of the suspension on a 100 mesh grid coated with nitrocellulose or carbon. The samples were usually shadowed with gold or chromium. The electron micrographs are shown in Figure 3.

Densities of single crystal preparations, dried at room temperature, were measured *via* flotation and density gradient columns.

## Interpretation of the Experimental Data

From the electron micrographs, it appears that the crystals have distinct oblong shape with a typical width of around 9000 Å and a length of the order of 22,000 Å. A particularly interesting feature of the crystals are the cracks which are invariably perpendicular to the long direction of the crystals. The larger cracks typically reveal fibers across the cracks. Some of the fibers are over 1000 Å in length and appear to be about 100 Å in cross section. Assuming the crystals to be of the same type as commonly found in polymers, with the chain direction approximately normal to the large faces, the drawn out fibers would have to correspond to as much as ten or more folds of the chain if they are extended fibers. It is also significant that all corners appear to be very nearly right-angled, which would indicate that the crystallographic unit cell might have an orthogonal base plane.

In examining the X-ray fiber pattern, a high degree of arching was noted. This may be attributed to the imperfect stacking of the single crystals as well as the possibility of the polymer chains being not exactly normal to the large face of the crystals. This type of arching of the reflections presents difficulty in assigning the correct layer lines for many of the reflections.

Table I lists all the *d* spacings which are greater than 3 Å and their apparent layer lines assignment. Two reflections appear to be either at or near the meridian on the second and fourth layer lines. The spacings correspond to 6.7 and 3.3 Å, respectively. Further examination of the pattern, taking into account all observed *d* spacings greater than 3 Å, leads to a layer-

(8) D. A. Rees, *J. Chem. Soc. B*, 217 (1969).
(9) N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 45, 85 (1969).
(10) S. A. Barker, E. J. Bourne, and D. H. Whiffen, *Methods Biochem. Anal.*, 3, 213 (1956).
(11) P. H. Giel, "Polymer Single Crystals," Interscience, New York, N. Y., 1963, p 79.

**Table I.** Calculated and Observed $d$ Spacings for Proposed Unit Cell for Mycodextran with $a$ = 17.6 Å, $b$ = 6.85 Å, and $c$ (fiber axis) = 13.4 Å

| Index | $d$ spacing, Å | | Intensity[a] |
|---|---|---|---|
| | Calcd | Obsd | |
| 101 | 10.66 | 11.30 | M |
| 200 | 8.80 | 8.77 | S |
| 201 | 7.35 | 7.37 | S |
| 002 | 6.70 | 6.69 | W |
| 011 | 6.11 | 6.14 | S |
| 111 | 5.77 | 5.88 | S |
| 301 | 5.37 | 5.34 | W |
| 211 | 5.01 | 5.00 | W |
| 012 | 4.79 | 4.77 | M |
| 311 | 4.23 | 4.34 | M |
| 212 | 4.21 | 4.15 | M |
| 013 | 3.74 | 3.84 | M–W |
| 203 | 3.98 | | |
| 500 | 3.52 | 3.58 | VS |
| 120 | 3.37 | 3.37 | M |
| 020 | 3.43 | | |
| 004 | 3.35 | 3.30 | VW |
| 104 | 3.29 | | |
| 403 | 3.13 | 3.24 | W |
| 313 | 3.15 | | |
| 510 | 3.13 | 3.19 | W |
| 220 | 3.20 | | |
| 204 | 3.13 | 3.07 | VW |
| 403 | 3.13 | | |

[a] S, strong; M, medium; W, weak; V, very.

line spacing of 13.4 Å along the fiber direction. Since the electron micrographs show the parallel faces of the crystals to be equivalent and the corners are right-angled, it is reasonable to assume the unit cell to have an orthogonal base plane. The reflections were indexed on the basis of a unit cell with dimensions $a$ = 17.6 Å, $b$ = 6.85 Å, and $c$ (fiber direction) = 13.4 Å. The calculated $d$ spacings are also listed in Table I.

The possible existence of meridional reflections on the second and fourth layer lines suggests the presence of a twofold screw axis along the chain. From the measured density of 1.480 ± 0.005 g/cc and the volume of the unit cell, the most probable number of anhydroglucose units per cell turns out to be 8. Exact agreement between observed and calculated densities would be achieved if there was one water molecule associated with each glucose unit. Indeed, the infrared spectrum of the sample exhibits a strong band at 1635 $cm^{-1}$, which is characteristic of absorbed water or water of hydration or both. Since the repeating unit in this case is a disaccharide, there should be four disaccharide units (or two chains) per unit cell. It is not necessary that these two chains should be related by a crystallographic symmetry operation. The appearance of the reflection with a spacing of 3.58 Å, which has been indexed as (500), rules out the possibility of a twofold screw axis parallel to the $a$ axis of the unit cell; hence the commonly occurring space group $P2_12_12_1$ cannot be assigned to this case. This leaves as possible space groups only $P22_12$ or $P222_1$. The former implies a twofold screw axis along the $b$ direction and this means two sugar residues would have to be placed in a distance of 6.85 Å, which is not possible. A final alternative would be the space group $P2_1$, with the unit cell monoclinic and the angle close to 90°.

Having derived the parameters characterizing the chain, namely the screw type and the fiber repeat, an attempt was made to arrive at the probable conformation of mycodextran using computer-based stereochemical techniques.

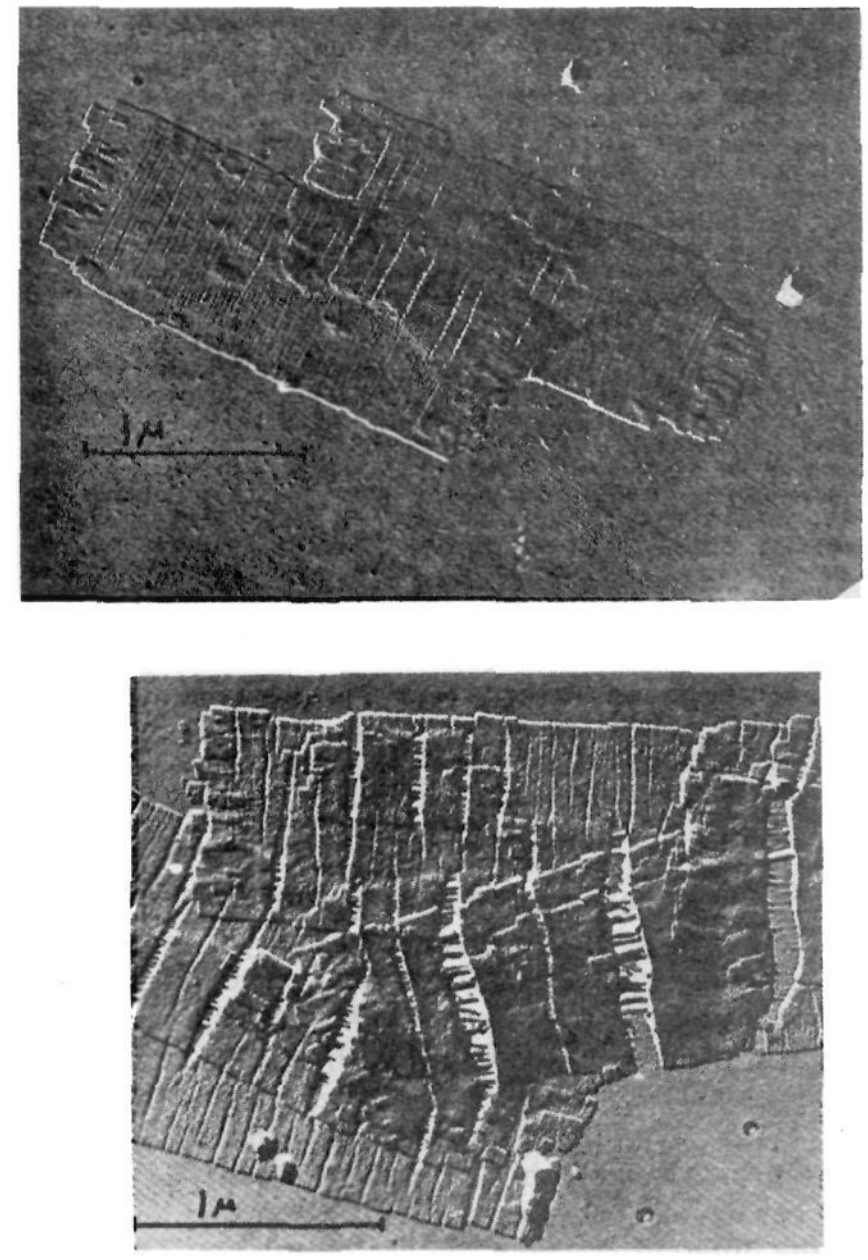



Figure 3. The electron micrographs of the single crystals of mycodextran.

**Conformational Analysis.** In assigning preferred conformations for polysaccharide chains, various workers[12–16] have used different functions to evaluate the nonbonded interaction energy.

In this paper, we applied the Kitaigorodsky functions of the form[17]

$$V(i,j) = 3.5[8600 \exp(-13Z) - 0.04/Z^6]$$

where $Z = r_{ij}/r_0$, $r_{ij}$ being the internuclear distance between the pair of nonbonded atoms $i$ and $j$, and $r_0$ is the equilibrium distance between them. The parameters $r_0$ used here are the same as used by Rao, *et al.*[12a]

Since experimental[18,19] and theoretical results[12a] have confirmed that the α-D-glucose residues in maltose and amylose exist in the $C1$ (chair) form, the same conformation was used in the calculations reported here.

(12) (a) V. S. R. Rao, P. R. Sundararajan, C. Ramakrishnan, and G. N. Ramachandran in "Conformation of Biopolymers," G. N. Ramachandran, Ed., Academic Press, London, 1967, p 721; (b) P. R. Sundararajan and V. S. R. Rao, *Biopolym.*, **8**, 313 (1969).
(13) A. Sarko and R. H. Marchessault, *J. Amer. Chem. Soc.*, **89**, 6454 (1967).
(14) D. A. Rees and R. J. Skerrett, *Carbohyd. Res.*, 7, 334 (1968).
(15) J. Blackwell, A. Sarko, and R. H. Marchessault, *J. Mol. Biol.*, **42**, 379 (1969).
(16) C. V. Goebel, W. L. Dimpfl, and D. A. Brant, *Macromolecules*, **3**, 644 (1970).
(17) A. I. Kitaigorodsky, *Tetrahedron*, **14**, 230 (1961).
(18) V. S. R. Rao and J. F. Foster, *Biopolymers*, **1**, 527 (1963).
(19) M. Rudrum and D. F. Shaw, *J. Chem. Soc.*, 52 (1965).

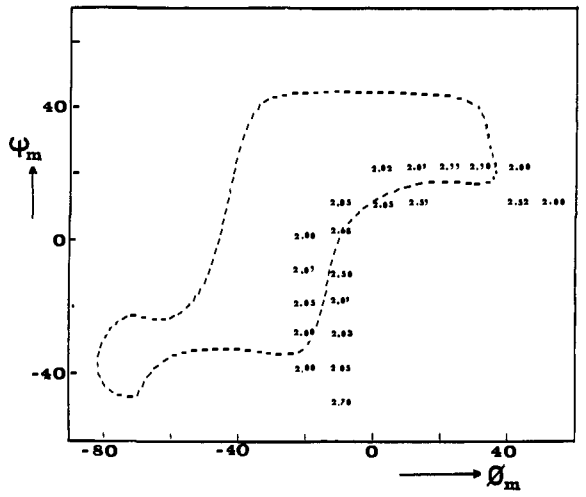



Figure 4. Extreme limit contour for a pair of D-glucose units joined *via* a $\alpha$-1→4′ linkage, as a function of ($\phi$, $\psi$). The O-2···O-3′ type of hydrogen bond distances are marked (taken in part from ref 12b).

The positional parameters for the atoms in the glucose residue have been taken as an average of those of the $\alpha$-D-glucose residues in the crystal structure of cyclohexaamylose.[20] A value of 119° was chosen for the angle at the bridge oxygens at both $\alpha$-1→4′ and $\alpha$-1→3′ linkages. The calculations were repeated with a value of 117.5° for these angles, and the results were not affected appreciably. Calculations carried out with the parameters for the residues, obtained as an average of the different crystal structure results for $\alpha$-D-glucose and its oligomers, do not change the results or conclusions. Hence, the data obtained from the former alone will be presented here.

While generating a polymer chain by the principle of rigid body transformation for conformational analysis, it is often the case that the geometrical repeating unit and the chemical repeating unit are identical. For example, in the case of the polysaccharides cellulose and amylose, the geometrical as well as the chemical repeating unit is the D-glucose residue. In the case of mycodextran, the polymer may be considered either as a polymaltose with an $\alpha$-1→3′ linkage along the chain or as a polynigerose with an $\alpha$-1→4′ linkage. Hence the geometrical (as well as the chemical) repeating unit may be either a maltose or a nigerose unit. The knowledge of the shape of the repeating unit is of prime importance in the analysis of polymer chain conformations. Since extensive studies have been reported in the literature on maltose,[12,15,16,20-23] it will be taken as the repeating unit in the present calculations.

As represented in Figure 1, a pair of maltose units joined through an $\alpha$-1→3′ linkage have freedom of rotations around the single bonds C-$1_n$–O and O–C-$3'_n$. The subscript n refers to the atoms in the glucose residues on either side of the $\alpha$-1→3′ linkage. Each one of the rotational states defines a conformation for the pair of repeating maltose units. Let these states be denoted by the conformational angles ($\phi_n$, $\psi_n$), $\phi_n$ defining the rotation around the C-$1_n$–O bond and $\psi_n$, that around the O–C-$3'_n$ bond. The subscript n denotes the nigerose type ($\alpha$-1→3′) linkage. The conformation corresponding to ($\phi_n$, $\psi_n$) = (0°, 0°) has been chosen similar to the definition of Sundararajan and Rao.[12b] Apart from this pair of rotational angles, free rotations are also possible for the glucose residues in maltose, around the C-$1_m$–O and O–C-$4'_m$ bonds. Let the conformations generated as a result of this pair of rotations be described by the angles ($\phi_m$, $\psi_m$), the subscript m denoting the maltose-type ($\alpha$-1→4′) linkage. Hence, it is possible to generate the mycodextran chain, as a function of ($\phi_n$, $\psi_n$), for each one of the conformations ($\phi_m$, $\psi_m$) assumed by the maltose unit. Thus, in this case, the geometrical (as well as the chemical) repeating unit itself possesses different conformational states, and this feature has to be taken into consideration in the analysis of copolysaccharide conformations, such as that of mycodextran.

Various methods of analysis have provided sufficient information on the shape of maltose. Stereochemical considerations have shown that the freedom of rotation of the glucose residues in maltose around the C-$1_m$–O and O–C-$4'_m$ bonds is restricted.[12,15,16] The conformational analysis also predicted an intramolecular hydrogen bond between O-$2_m$ and O-$3'_m$ hydroxyls of the contiguous residues.[12b,15,16] The crystallographic, infrared, and nmr studies[20-23] have confirmed the presence of such a hydrogen bond in maltose. The extreme limit contour (based on the contact distance criterion[24]) for a pair of 1→4′ linked $\alpha$-D-glucose residues is given in Figure 4, as a function of the conformational angles ($\phi_m$, $\psi_m$). The conformations which facilitate the above-mentioned hydrogen bond are also marked in this figure. Because of evidence in support of this type of intramolecular hydrogen bond in maltose and amylose, in the present calculations on mycodextran it was considered that the O-$2_m$···O-$3'_m$ type of hydrogen bonding occurs in this polymer as well. On this criterion, the number of conformational states for maltose in mycodextran is limited to those ($\phi_m$, $\psi_m$) in Figure 4, which allow the hydrogen bond. This amounts to considering about 20 different conformational states for the repeating (maltose) unit in mycodextran, if both $\phi_m$ and $\psi_m$ are varied at intervals of 10°.

The maltose unit with a particular value of ($\phi_m$, $\psi_m$) was generated.[25] The disaccharide (which is the repeating unit) was then projected onto a standard coordinate system of axes, with O-$3_n$ as origin, O-$1'_n$ on the $y$ axis and O-$3'_n$ O-$1'_n$ and C-$1'_n$ defining the $XY$ plane as shown in Figure 5. The 1→3′ linked dimer was obtained by means of rigid body transformations, corresponding to ($\phi_n$, $\psi_n$) = (0°, 0°).[25]

With the conformation of the repeating maltose unit thus being in a state ($\phi_m$, $\psi_m$), the dihedral angles ($\phi_n$, $\psi_n$) were varied from 0 to 360°, each at intervals of 10°, and the nonbonded interaction energy was evaluated for the tetrasaccharide. The helical parameters $n$,

(20) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, 87, 2779 (1965).
(21) S. S. C. Chü and G. A. Jeffrey, *Acta Crystallogr.*, 23, 1038 (1967).
(22) G. J. Quigley, A. Sarko, and R. H. Marchessault, *J. Amer. Chem. Soc.*, 92, 5834 (1970).
(23) B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Tetrahedron*, 22, 3061 (1966).
(24) G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, in "Aspects of Protein Structure," G. N. Ramachandran, Ed., Academic Press, London, 1963, p 121.
(25) P. R. Sundararajan, Ph.D. Thesis, University of Madras, India, 1969.

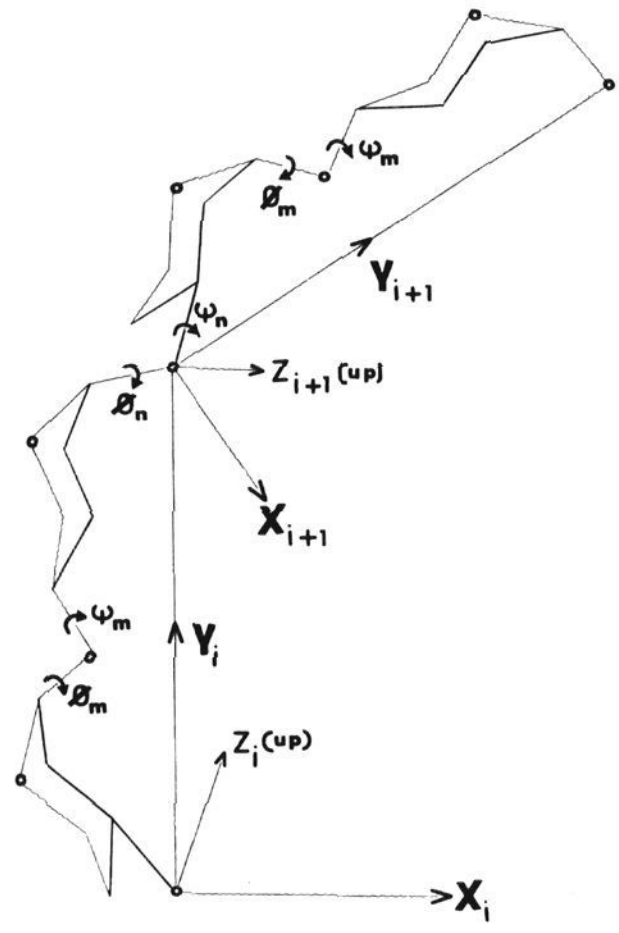



Figure 5. Coordinate system of axes used to correlate the repeating units $i$ and $(i + 1)$ along the mycodextran chain. The repeating (maltose) unit is in a state $(\phi_m, \psi_m)$.

the number of repeating units per turn, and $h$, the unit translation, were also calculated.

This was repeated for each one of the conformations $(\phi_m, \psi_m)$. A three-dimensional view of the results is given in Figure 6. In this figure, each one of the layers corresponds to one maltose state. The energy contour of 0 kcal/mol is given on these layers as a function of $(\phi_n, \psi_n)$. The "allowed volume" is only a small fraction of the entire conformational volume. On each layer, the small circles represent the positions of minimum nonbonded interaction energy for that $(\phi_n, \psi_n)$ surface. There are two positions on each $(\phi_n, \psi_n)$ surface at which a chain with parameters $n = 2$ and $h = 6.7$ Å (corresponding to a repeat of 13.4 Å) is possible. Of these, one of them occurs within the permissible area while the other occurs at a region of high nonbonded interaction energy. If we imagine a curve to be traced through all the points marked 0, it represents the locus of the positions of minimum energy and a similar curve can be drawn through the points marked by X, representing the positions of the twofold chain. The layer on which these two curves pass closest to each other is the most favorable conformation in terms of $(\phi_m, \psi_m)$ and $(\phi_n, \psi_n)$. This occurs in the present case for a value of $(\phi_m, \psi_m) = (-10°, -40°)$ and $(\phi_n, \psi_n) = (-5°, -50°)$.

Figure 7 shows the curves of iso $n$ and iso $h$ as a function of $(\phi_n, \psi_n)$ for the state with $(\phi_m, \psi_m) = (-10°, -40°)$. The iso energy curves are also presented here. It is interesting to note that the position A, which represents the $2_{6.7}$ chain (*i.e.*, two residues per turn with a unit translation of 6.7 Å along the helix axis), is very close to the position of minimum energy (M). This map also shows that values of $n$ greater than 3 and $h$ less than 4 Å are not favored from energy considerations. This indicates that the mycodextran chain prefers an extended conformation.

Figure 6. A three-dimensional view of the 0 kcal/mol energy surface. Each layer represents a maltose in a $(\phi_m, \psi_m)$ state and the curve of 0 kcal/mol is drawn on each layer, as a function of $(\phi_n, \psi_n)$: (○) positions of minimum energy, (×) positions of the chain with $n = 2$ and $h = 6.7$ Å.

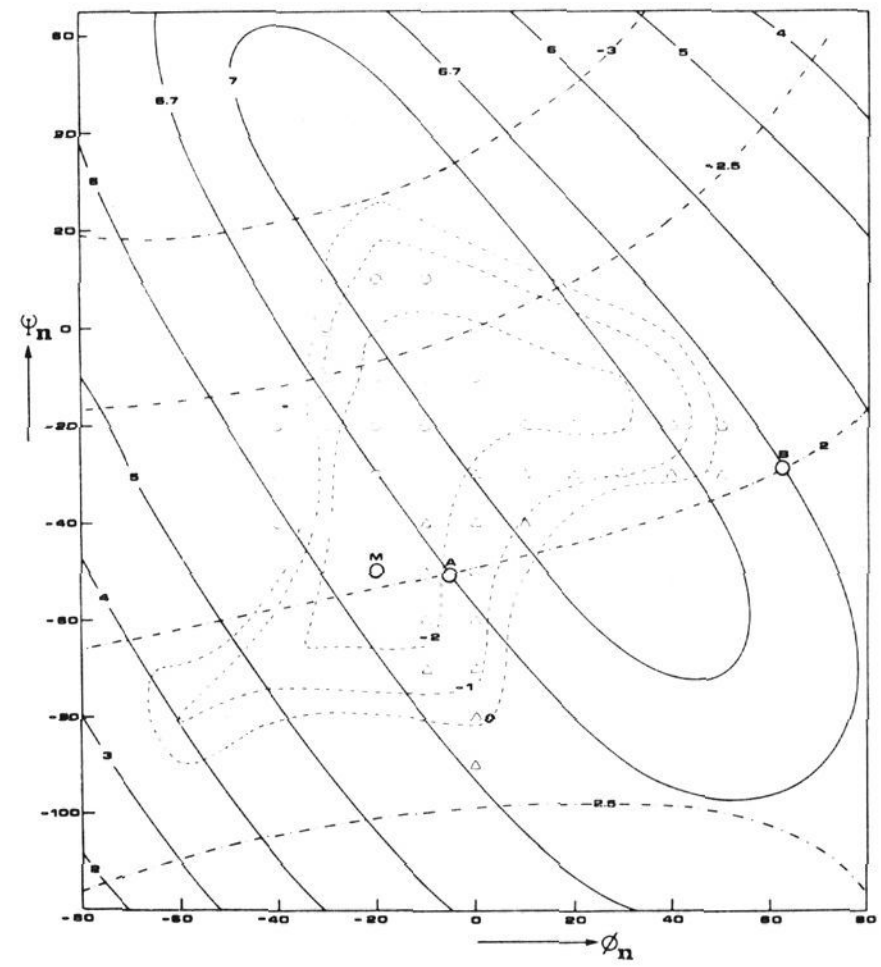



Figure 7. Curves of iso $h$ (——) and iso $n$ (–·–·–) as funcitons of $(\phi_n, \psi_n)$, for the state $(\phi_m, \psi_m) = (-10°, -40°)$. The iso energy curves (– – – –) are also given here. Conformations which favor the O-$5_n$···O-$4_n'$ type of hydrogen bonds (○) and the O-$2_n$···O-$4_n'$ type of hydrogen bonds (Δ) are shown: (M) minimum energy position, (A, B) positions of the chain with $n = 2$, $h = 6.7$ Å.

Figure 7 also shows that two types of intramolecular hydrogen bonds are possible between the contiguous

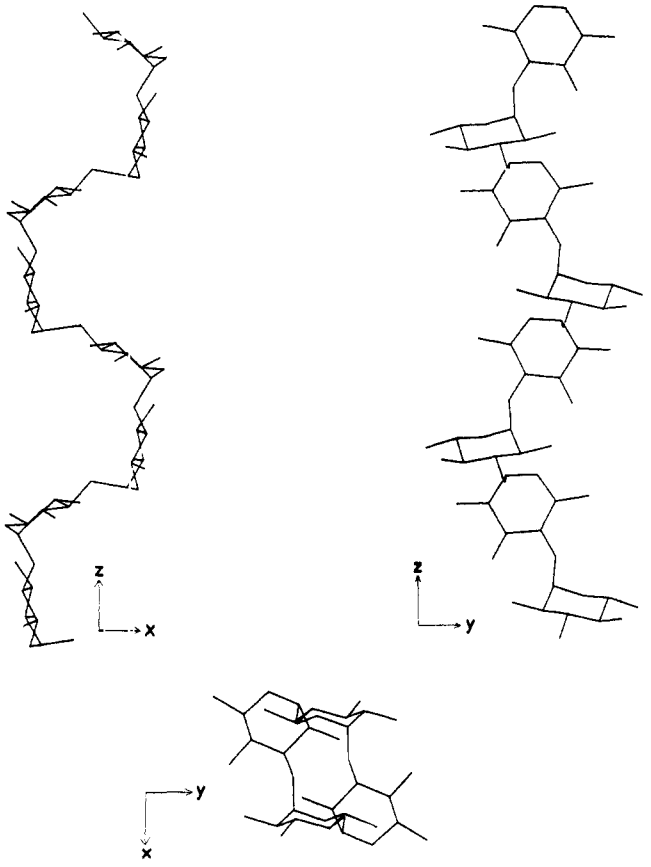



Figure 8. The proposed conformation of the mycodextran chain in (a) *X–Z*, (b) *Y–Z*, and (C) *X–Y* projections. The chain axis is along the *Z* axis.

glucose residues in the α-1→3′ linkage. One of them is a hydrogen bond between the O-$5_n$ atom and the O-$4'_n$ hydroxyl group, whereas the second type is between the O-$2_n$ and the O-$4'_n$ hydroxyl groups. At the position where the $2_{6,7}$ chain occurs, however, only the latter type is possible.

Table II gives the range of values of ($\phi_m$, $\psi_m$) examined and the possible intramolecular hydrogen bonds. It is seen that of all the values of ($\phi_m$, $\psi_m$) which favor the O-$2_m\cdots$O-$3'_m$ hydrogen bonding in maltose, only three of the conformations give rise to the additional O-$2_n\cdots$O-$4'_n$ hydrogen bond between the 1→3′ linked contiguous residues. The conformation with ($\phi_m$, $\psi_m$) = (−10°, −40°) is preferred on both nonbonded interaction energy and hydrogen bond criteria. Hence, the most probable conformation of mycodextran possesses two types of intramolecular hydrogen bonds: (i) O-$2_m\cdots$O-$3'_m$ of length 2.63 Å between the α-1→4′ linked glucose units and (ii) O-$2_n\cdots$O-$4'_n$ of length 2.95 Å between the α-1→3′ linked glucose units. Three orthogonal perspectives of the mycodextran molecule in this conformation are shown in Figure 8.

**Table II.** List of ($\phi_m$, $\psi_m$) Examined and the Resulting Hydrogen Bond Types and Their Lengths[a]

| ($\phi_m$, $\psi_m$), deg | ($\phi_n$, $\psi_n$), deg | O-$2_m\cdots$ O-$3'_m$, Å | O-$2_n\cdots$ O-$4'_n$, Å |
|---|---|---|---|
| (−20, −40) | (−20.4, −42.6) | 2.99 | |
| (−20, −30) | (−20.5, −48) | 2.89 | |
| (−20, −20) | (−21.8, −52.6) | 2.85 | |
| (−20, −10) | (−23.8, −56.8) | 2.87 | |
| (−20, 0) | (−26.7, −60.3) | 2.96 | |
| (−10, −50) | (−4.3, −43) | 2.78 | 2.88 |
| (−10, −40) | (−5.3, −50.3) | 2.63 | 2.85 |
| (−10, −30) | (−7.7, −56.6) | 2.53 | 2.95 |
| (−10, −20) | (−11.2, −61.8) | 2.51 | |
| (−10, −10) | (−15.8, −66) | 2.56 | |
| (−10, 0) | (−20.8, −69.6) | 2.68 | |
| (−10, 10) | (−26.5, −72.5) | 2.85 | |
| (0, 10) | (−22.9, −80.9) | 2.65 | |
| (10, 10) | (−19.4, −88.6) | 2.51 | |
| (10, 20) | (−28.6, −91) | | |

[a] The ($\phi_n$, $\psi_n$) values given here correspond to $n$ = 2 and $h$ = 6.7 Å.

**Virtual Bonds *vs.* Real Bonds.** The virtual bond approach for conformational analysis of polysaccharides was introduced by Jones.[26] For the cellulose case, which he considered, the virtual bond is the end-to-end vector of the repeating unit; *i.e.*, it joins the O-1 and O-4 atoms of each glucosidic unit. This method requires prior knowledge of $n$ and $h$; it then explores through the locus of points in conformational space corresponding to a fixed $n$ and $h$ by rotation of the residue around the virutal bond which consequently varies the angle at the bridge oxygen ($\tau$). If one adds a third dimension to the $\phi$, $\psi$ map (Figure 7), corresponding to the variable $\tau$, then the virtual bond approach explores along two loci which pass through the two $2_{6,7}$ points in the $\phi$, $\psi$ plane of Figure 7. Clearly this is a very limited region compared with the overall $\phi$, $\psi$ space. It has the advantage, however, of exploring in terms of the angle $\tau$, and one must admit that, ideally, conformational maps of the Figure 7 type should be three dimensional with $\phi$, $\psi$, and $\tau$ being varied systematically.

In at least one case, β-D-(1→4′)-xylan where both the virtual bond[27] and conformational map[28] approach have been used, the interpretation of the X-ray data *via* the two approaches coincided, but the latter provided much more widely useful information in terms of overall chain properties, particularly those of a statistical nature.[29]

The virtual bond treatment was applied to the case of mycodextran, by taking the vector joining O-$3_m$ and O-$1'_m$ atoms (coinciding with the $Y_i$ direction in Figure 5) as the virtual bond. Similar to the procedure mentioned above, the maltose conformation was chosen such that the O-$2_m\cdots$O-$3'_m$ hydrogen bond is preserved. It was found that for a value of $\tau$ = 117.5°, while the O-$2_m\cdots$O-$3'_m$ hydrogen bond was strong, of the order of 2.7 Å in length, the O-$2_n\cdots$O-$4'_n$ distance was rather large, of the order of 3.1 Å. If the value of $\tau$ is increased to a range of 118–120°, the latter distance also becomes acceptable in terms of hydrogen bonding, being about 3.0 Å in length. This justifies the value of 119° chosen for $\tau$ for the analysis in terms of ($\phi$, $\psi$), discussed in the earlier sections. The virtual bond analysis was carried out with the parameters for the glucose residues, derived as an average of different crystal structure analyses on α-D-

(26) D. W. Jones, *J. Polym. Sci.*, **32**, 371 (1958).
(27) W. J. Settineri and R. H. Marchessault, *J. Polym. Sci., Part C*, **11**, 253 (1965).
(28) P. R. Sundararajan and V. S. R. Rao, *Biopolymers.*, 8, 305 (1969).
(29) V. S. R. Rao, N. Yathindra, and P. R. Sundararajan, *ibid.*, **8**, 325 (1969).

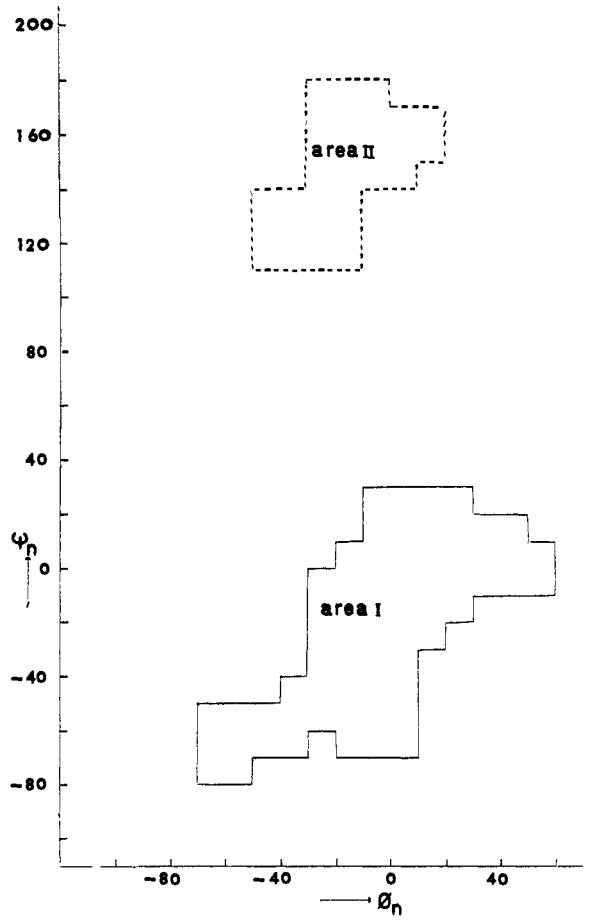



Figure 9. Regions of allowed conformations of ($\phi_n$, $\psi_n$) for a state with ($\phi_m$, $\psi_m$) = (−10°, −40°). Conformations in area II give rise to minor close contacts (see text).

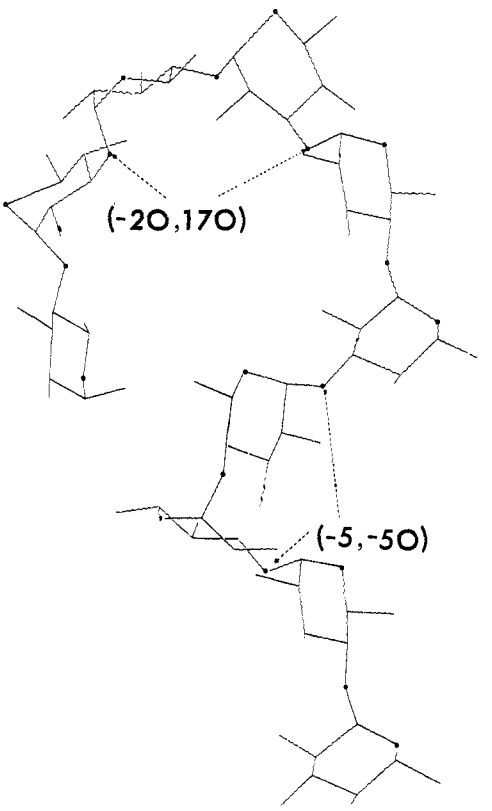



Figure 10. A view of the folded mycodextran chain. The portion of the chain on the right side of the fold has the regular twofold screw axis. The ($\phi_n$, $\psi_n$) has been made different at two successive α-1→3′ linkages. The axis of the chain with the regular twofold screw axis is in an arbitrary direction.

glucose and its oligomers. Though the above calculation demands the value of $\tau$ to be in the range of 118–120° for the two types of hydrogen bonds to be formed, it was found that if the parameters for the α-D-glucose unit as derived from the crystal structure of cyclohexaamylose are used, a value of $\tau$ = 117.5° will be sufficient to achieve both types of hydrogen bonds. This agrees with a recently published study on α-glucans which included mycodextran.[30] Differences in the values of $\phi_n$, $\psi_n$ reported in that study compared with this report are most probably due to slight differences in the definition of the starting conformation, *i.e.*, $\phi$, $\psi$ = 0°, 0°. Another source of discrepancy in comparing results is due to different starting coordinates for the glucose residue. This factor is all the more important when dealing with a large repeating unit as in a regular copolysaccharide.

**Chain Folding.** The phenomenon of chain folding is common in polymer single crystals. The fiber diagram in Figure 2 indicates that the chains run normal to the surface of the single crystals and, since small angle X-ray measurements demonstrated that these were about 100 Å in thickness, it is reasonable to conclude that some amount of chain folding is present. Though several hypotheses exist on the chain-folding process for polysaccharides,[30,31] there is still a great lack of understanding of the phenomenon for these systems. In this section, an attempt has been made to indicate, though speculatively, how the mycodextran chain could fold.

As was discussed earlier, in the observed structure, mycodextran prefers an extended conformation with $n$ = 2 and a repeat of 13.4 Å. Examining Figure 7, one deduces that conformations with $n$ greater than 3 and $h$ less than 4 Å are not favored. This is in contrast to the case of the amylose chain (to which mycodextran has significant chemical similarity) which prefers flat helical structures with small $h$ and large $n$.

Careful analysis of the ($\phi$, $\psi$) map of the mycodextran chain reveals that in addition to area I in Figure 9, which is similar to the zone in Figure 7, a rather small area (area II) of ($\phi$, $\psi$) also corresponds to a sterically possible rgion. In this region, the C-$1_n$ and C-$4'_n$ atoms of contiguous residues are about 2.8 Å apart, which is only 0.2 Å below the extreme limit.[24] However, such minor close contacts might be expected to be tolerated in a polymer structure or they might be relieved by a slight change in the geometry of the repeating unit. In area II, the number of residues per turn varies from 6 to 9 and the value of $|h|$ is smaller than 3 Å. Hence the chain conformations with the ($\phi$, $\psi$) in this region are likely to be flat helices like the amylose chain. A conformation with the ($\phi$, $\psi$) corresponding to area II is thus prone to make the chain bend around and enable it to fold.

A computer program was constructed[25] such that, given the geometrical parameters of the repeating unit,

(30) B. K. Santhyanarayana and V. S. R. Rao, *Biopolymers.*, **11**, 1379 (1972).

(31) A. Sarko and R. H. Marchessault, *J. Polym. Sci., Part C*, **28**, 317 (1969).

the type of linkage and the angle at the bridge atom, a chain with any required degree of polymerization could be generated, with any desired values of $\phi$'s and $\psi$'s between the residues. This program enables one to generate a polymer chain, which could be nonhomogeneous in terms of the conformational angles ($\phi$, $\psi$). Figure 10 depicts the mycodextran chain so generated, in which the ($\phi_n$, $\psi_n$) at two of the $\alpha$-1→3′ linkages along the chain has been chosen from area II. The figure shows that the perturbation at two adjacent linkages is sufficient to achieve the folding. The segments of the chain on either side of the fold are about 11 Å apart.

The packing of chains and the question of chain polarity in the unit cell must await the obtaining of superior X-ray data than were available to the writers.

## Conclusions

Biopolymers of the mycodextran type, *i.e.*, with a regular copolymeric structure, are common both in plant and bacterial systems. The possible number of such regular molecular types based on permutations among the commonly occurring sugars, the glycosidic linkage type, and linkage configuration is exceedingly large. So far researchers have concentrated on homopolysaccharides, and this is only the second alternating copolysaccharide whose crystalline conformation is reported.[32] Nevertheless, the combined X-ray and conformational analysis approach as described herein would seem to offer the potential to derive the reference conformation which is the most likely candidate for being the maximum population state in the living system. It remains to be seen whether interaction with proteins, particularly in the enzyme–substrate complex, always involves this conformer or something closely akin. In the lysozyme–chitin case,[33] the conformation of the polysaccharide closely resembles that of the crystalline substrate. Mycodextranase rapidly degrades mycodextran to a tetramer,[34] which is the crystalline repeating unit. Subsequent hydrolysis is much slower and only proceeds to the disaccharide, nigerose. Furthermore, while the enzyme is specific for the $\alpha$-1→4 link in mycodextran, it does not attack the same linkage in amylose. It has been suggested that enzyme action on polysaccharide substrates is dictated by the structure of the glycosyl moiety that becomes the reducing end unit of the product liberated.[35] If the maltose conformation in crystalline mycodextran is the same as in amylose, as we conclude, then it is clear that at least a glycosyl disaccharide conformation ($\alpha$-1→3 unit in this case) is involved in triggering the enzyme action as has been suggested by the enzymologists[34] and would seem to be confirmed by the conformational data of this paper.

**Acknowledgments.** This work was partially supported by the National Research Council Canada.

(32) N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, **45**, 85 (1969).

(33) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, 167, 378 (1967).

(34) E. T. Reese and M. Mandels, *Can. J. Microbiol.* 10, 103 (1964).

(35) A. S. Perlin and S. Suzuki, *Can. J. Chem.*, **40**, 50 (1962).

# *Communications to the Editor*

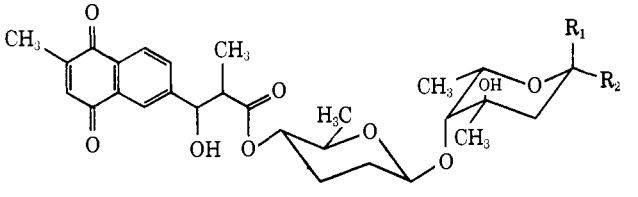
## Axenomycins. I. The Structure of Chromophore and Sugar Moieties

*Sir:*

Axenomycin A, B, and D represent the major components of a new group of closely related antibiotics which are produced by *Streptomyces lysandri n. sp.* and have a complex structure containing a macrocyclic lactone, two sugar residues, and a 1,4-naphthoquinone chromophore. They have activity against plateworms and yeasts. We report here the structure of the chromophore and sugar moieties of axenomycin B, which are common to all the components of this group.

Axenomycin B, a neutral, crystalline substance, $C_{78}H_{126}O_{30}$ (mol wt 1530 (calcd 1542);[1] $\lambda_{max}$(MeOH) 250, 256, and 267 nm; $\nu_{quinone}$ 1665 cm$^{-1}$), on chromic acid oxidation[2] afforded 2-methyl-1,4-naphthoquinone-6-carboxylic acid[3] (methyl ester, mp 160°), identified by direct comparison with a synthetic specimen prepared from 2-methyl-6-naphthoic acid,[4] thus revealing the substitution of the naphthoquinone chromophore.

Methanolysis (0.05 *N* HCl at room temperature) of axenomycin B gave, in addition to axenolide,[5] **1a** ($\alpha$-



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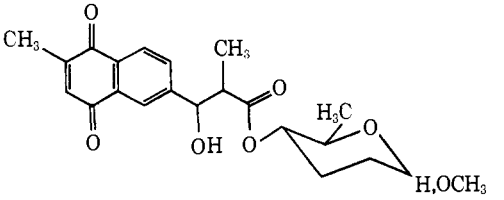

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(1) Determined by the vapor pressure method.

(2) D. W. Mac Corquodale, L. C. Cheney, S. B. Binkley, W. F. Holcomb, R. W. Mc Kee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **131**, 357 (1939).

(3) All the compounds have consistent elemental analyses and spectroscopic properties. Melting points, uncorrected, were taken at the Köfler hot stage. Unless otherwise stated optical rotations were measured in chloroform at 20°.

(4) G. A. R. Kon and W. T. Weller, *J. Chem. Soc.*, 792 (1939); L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed, D. C. Heath Co., Boston, Mass., 1941, p 233.

(5) F. Arcamone, G. Franceschi, B. Gioia, S. Penco, and A. Vigevani, *J. Amer. Chem. Soc.*, **95**, 2009 (1973).