proton at C_2 in the lysine moiety occurs at δ 3.70, whereas the proton at C_2 in the glutamic acid moiety occurs as a multiplet at δ 4.0 – 4.3 (corresponding value for pyroglutamic acid δ 4.0 – 4.3 ppm). These spectroscopic data lend further support to the structures of pyrosaccharopine and saccharopine.

Experimental. Synthesis of a diastereoisomeric mixture of L-*saccharopine and* D-*allosaccharopine.* A solution of 2-ketoglutaric acid (0.05 mol) and KCN (0.32 mol) in 1 N NaOH (100 ml) and adjusted to pH 11.4 with HCl was mixed with a solution of L-lysine, HCl (0.05 mol) in 1 N NaOH (75 ml), adjusted to pH 11.4 with NaOH. The mixture was kept at 50° for 22 h. Conc. hydrochloric acid (330 ml) was added, and the solution was refluxed for 18 h. The reaction mixture was concentrated to dryness. The residue was dissolved in water, and the amino acid fraction was isolated by binding to a strongly acid ion-exchange resin in the hydrogen form, followed by elution with ammonia. The individual amino acids were separated by ion-exchange chromatography on a strongly basic ion-exchange resin (Dowex 1 × 8, 200 – 400 mesh, 3 × 77 cm) in the acetate form, by elution with acetic acid (1 N). Fractions of 20 ml were collected. Lysine occurred in fractions Nos. 10 – 13, saccharopine in fractions Nos. 38 – 47, and pyrosaccharopine in fractions Nos. 65 – 78. The fractions containing saccharopine were evaporated to dryness. Crystallization from aqueous ethanol gave a paperchromatographically homogeneous mixture of the two diastereoisomers (2.6 g, 19 %), $[\alpha]_D^{20}$ +15° (*c* 1.9, 0.5 N HCl).

Synthesis of L-*pyrosaccharopine (*(5S, 5'S)-N-*(5-amino-5-carboxypentyl)-2-pyrrolidon-5-carboxylic acid).* L-Saccharopine of natural origin (262 mg) was refluxed in water (20 ml) for 5 h. Evaporation to dryness, and two recrystallizations from water-acetone afforded an analytical sample. (Found: C 50.65; H 7.17; N 10.79. Calc. for $C_{11}H_{18}O_5N_2$: C 51.15; H 7.03; N 10.85.) $[\alpha]_D^{23}$ +3.4° (*c* 1, H_2O), $[\alpha]_D^{21}$ +7.7° (*c* 1, 1 N HCl). M.p. 164 – 167° (decomp.) (determined by inserting the sample in an oil bath preheated to 260°).

Rotations were determined in a 1 dm tube. NMR-spectra were measured on a JEOL C-60 HL instrument. TMS was used as an internal standard in trifluoroacetic acid, sodium 3-trimethylsilyl-2,2',3,3'tetradeuteriopropionate in deuterium oxide. Microanalyses were performed by Mr. G. Cornali and his staff.

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The Molecular Basis for Some Physical Properties of Polyuronides

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The previous experimental work on the solution properties of alginate [1,2] has mostly dealt with one alginate sample prepared from *Laminaria digitata* with a ratio of 1.6 between D-mannuronic acid (M.A.) and L-guluronic acid (G.A.) residues. Alginate contains [3-6] long homopolymeric blocks of each monomer, together with blocks of the alternating sequence. In this work we investigate samples containing different amounts of the three types of structure, and we try to estimate their relative extension in the unperturbed state. Ion exchange experiments [7] have shown that in the gel state L-guluronic acid has a markedly higher affinity for calcium ions than has D-mannuronic acid. We shall here compare the ion exchange behaviour of the three types of blocks at conditions where they are all soluble. We then discuss the results in terms of the molecular structure of the polymer chains. The D-mannuronic and L-guluronic acids have

Table 1. Characterization of sequence and extension of some alginate samples.

% G.A.	"MG-blocks"	"MM-blocks"	"GG-blocks"	B	K_θ × 10^3	C_∞[a] Å	C_∞[b] Å
9	9.1	79	8	0.040	11	23	24
72.5	25	0	75	0.031	15	27	37
38.5	30	41	29	0.040	11	23	27
35.5	65	25	10	0.065	5.8	16	19

[a] Calculated from K_θ with the assumption of C1-conformation of both M. A. and G. A.
[b] Calculated with the assumption of C1 for M. A. and 1C for G. A.

Table 2. Estimation of the number of "fully allowed"[17] conformation in dimers. The total number of rotational isomers is $36^2 = 1296$. The details of the calculation are given in papers by Whittington.[18,19]

Dimer residue	MM	MG	GM	GG
Both M. A. and G. A. in C1	14	42	14	42
M. A. in C1 and G. A. in 1C	14	22	2	7

been shown to exist in the C1 ($^4C_1$) and the 1C ($^1C_4$) conformation, respectively, in the acid crystalline form.[8,9] Their ring conformation in solution is not known. From consideration of the distribution of bulky groups among the equatorial and axial positions in the ring we regard the C1-conformation to be the most probable for the D-mannuronic acid residues. The ring conformation of the L-guluronic acid residues is less clear, and we shall here consider both the C1 and the 1C conformation in the discussion.

Results and discussion. Light scattering results on alginates of different composition have suggested [10] that at ionic strength 0.1 the relative extension of the three types of blocks increases in the order

"MG-blocks" < "MM-blocks" < "GG-blocks"

Their relative extension in the unperturbed state was determined from viscometrical B-values [11,12] of alginate samples of different composition and sequence.[3-6] The results are given in Table 1 together with some derived [11] parameters for unperturbed chain dimensions.

The results in Table 1 indicate that the relative extension in the unperturbed state of the three types of blocks increases in the order

MG-blocks < MM-blocks < GG-blocks

Calculations [13] of the free rotation dimensions of several 1,4-linked polysaccharides yielded C_∞-values which were from 1/10 to 1/20 of the C_∞-values given in Table 1. This suggests that the rotation around all the different types of glycosidic linkage must be severely restricted and that the reason for the difference in the relative extension of the blocks must be a difference in the hindrance to rotation around the glycosidic linkages. We have here used "hard-sphere" calculations [14] for estimating the hindrance to rotation in the different dimer residues in alginate. This type of calculation has been shown by Whittington [15] to yield results which are in qualitative agreement with statistical mechanical calculations.[16] In Table 2 are given results from such calculations.

Table 2 indicates, in agreement with experiments, that the rotation around the two single bonds of the glycosidic linkage is severely restricted. However, it is only when the ring conformation is 1C for L-guluronic acid that the hindrance to rotation is higher in the GG-dimer than in the MM-dimer. Whittington [19] has shown that in a polymer of the alternating sequence the high local flexibility in the

MG-dimer (G.A. as the reducing end) dominates its overall flexibility yielding chain dimensions which is lower than that of polymannuronic acid. The experimental order of flexibility among the three types of blocks is, therefore, qualitatively explained [10] by these considerations about steric interaction when the L-guluronic acid is kept in the 1C-conformation.

Ion exchange experiments on alginate fragments enriched in the different type of monomer sequence were carried out for the Ca-Mg exchange reaction as previously described [7] with results as shown in Fig. 1.

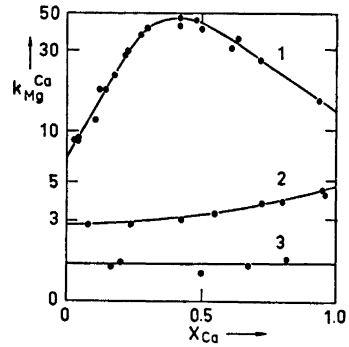

Fig. 1. The selectivity coefficient, k_{Mg}^{Ca}, for alginate fragments as a function of the fraction, X_{Ca}, of calcium bound to the polyelectrolyte. Curve 1: 90 % G. A., Curve 2: Alternating fragment (38 % G. A.), Curve 3: 90 % M. A.

All three fragments were shown by centrifugation experiment to be soluble at low contents of calcium bound to the fragments. The order of selectivity, MM-blocks < MG-blocks < GG-blocks, obtained at $X_{Ca} = 0$ is thus a property of the fragments in their soluble state. The D-mannuronic acid and L-guluronic acids are C_5-epimers and if both acids adopt the C1-conformation the carboxyl groups are equatorial and axial, respectively.

Inspection of molecular models gives in this case no obvious reason for such a difference in selectivity.

If the L-guluronic acid adopts the 1C-conformation the glycosidic linkage becomes diaxial and the position of the rings relative to each other is now very different from that of the D-mannuronic acid dimer residue. Model building shows that a "cavity" with very hydrophilic inner surface is formed between the rings. The cavity consists of the carboxyl group in the reducing end (marked CX′ in Fig. 2) and of the ring oxygen (O_5'), the bridge oxygen (O_1) and the two hydroxyl groups in the non-reducing end (O_2 and O_3). The cavity is facing out behind the plane in Fig. 2 where a dimer residue of L-guluronic acid in the two chair conformations is seen.

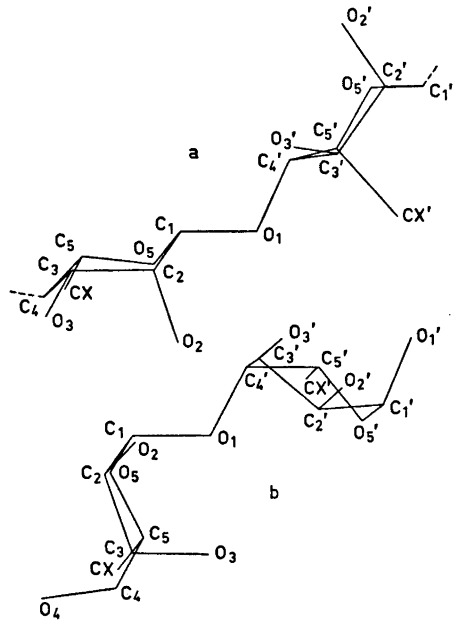

Fig. 2. Projections of L-guluronic acid dimer residues in the two chair conformations. a. C1-conformation. b. 1C-conformation.

Fig. 2 is a projection of the dimers as obtained from the computer when the torsion angles of the two single bonds of the glycosidic linkage are such that no overlap of van der Waals radii occurs. The range of "fully allowed"[17] torsion angles is so narrow that the distance between atomic groups of adjacent monomers varies between relatively narrow limits. The distance between the center of the carboxyl group (marked with CX in the figure) of adjacent rings is between 6.2 – 7.3 Å and 4.7 – 5.7 Å for the C1 and the 1C conformation, respectively. The $COO^- - COO^-$ distance in polymannuronic

acid is similar to that in polyguluronic acid in the C1 conformation, and the possibility must be considered that the difference in selectivity of the two polymers might be caused by a difference in charge density. We have, however, previously shown that polyelectrolytes with a higher charge density than this have no significant selectivity in the Ca-Mg-exchange reaction.[20] In addition we have shown that polyguluronic acid with 42 % of its carboxyl groups being reduced still has a k_{Mg}^{Ca} value as high as 6 in the limit of very low X_{Ca}-values.[21]

Instead, we suggest that the binding energy of calcium ions is lowered as a result of interaction with one or more oxygen atoms in the cavity shown in Fig. 2b. Calculations of $COO^- - O$ distances in the "fully allowed" range of torsion angles indicate that a calcium ion may simultaneously contact the carboxyl group in one residue and the two oxygens of the hydroxyl groups in the preceding residue. In addition the possibility of interaction with the ring oxygen and the bridge oxygen exists. Neutralization of the other charge of the calcium ion from direct contact with a neighbouring carboxyl group is not possible because this carboxyl group is situated on the other side of the sugar ring. A non-specific neutralization by the surrounding carboxyl groups must, therefore, be suggested.

The situation in the alternating sequence is less clear, but it is only when the L-guluronic acid residues are kept in the 1C conformation that the $COO^- - O$ distances are intermediate to those of the homopolymers.

In the discussion, it has so far been assumed that the high selectivity of polyguluronic acid is due to calcium binding to isolated chain molecules. There are, however, indications that magnesium alginate forms some aggregates in solution.[22,23] Neutralization of the "other" charge of the calcium ion might possibly, therefore, also occur from a carboxyl group of another polymer molecule even in the limit of very low X_{Ca}-values. At higher X_{Ca}-values, in the gel state, an autocooperative interchain contact is most probably the cause of the strong Ca-binding.[21] The position of the calcium ions in such structures is unknown, but it may tentatively be suggested that the binding site discussed here may constitute part of the binding site also in this case.

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