X-RAY AND INFRARED STUDIES ON CARRAGEENIN*

by

S. T. BAYLEY

Division of Applied Biology, National Research Laboratories, Ottawa (Canada)

INTRODUCTION

Chemical evidence[1] suggests that carrageenin, the polysaccharide extracted from the red algae *Chondrus crispus* and *Gigartina stellata*, consists mainly of D-galactopyranose residues joined by α-1,3-glycosidic linkages and that the majority of these residues have an ester sulphate on carbon 4. Small amounts of L-galactose, glucose and xylose have also been detected, although SMITH *et al.*[7] regard all three as impurities. JOHNSTON AND PERCIVAL[3] have obtained chemical evidence for branching. COOK *et al.*[4] reported that the molecular weight of carrageenin lay in the range 100,000–500,000 and distinguished two components from their sedimentation measurements. Later SMITH AND COOK[5] discovered that one of these components, designed κ-carrageenin, is sensitive to potassium ions while the other, λ-carrageenin, is not and showed that they differ in sulphate content and optical rotation. Recent analyses[6,7] have established the chemical composition of the two components with fair precision and shown that while λ-carrageenin is largely a sulphated poly-D-galactan, more than one third of the residues in the κ-component are 3,6-anhydro-D-galactose.

The present work was undertaken in an attempt, from X-ray diffraction and infrared absorption studies, to characterize these two fractions further and to examine their structural relationship in whole carrageenin.

MATERIALS AND METHODS

Materials

The materials studied were prepared as the sodium salts from the same high viscosity commercial samples, CM1 and C5, investigated by SMITH *et al.*[2,7]. These samples were probably obtained entirely from *Chondrus crispus*. In the present investigation, no differences could be detected between them. The molecular weight of whole carrageenin (sample C5) was 358,000[2].

The κ- and λ-components were obtained in almost equal yields from whole carrageenin by methods previously described[2,5]. The molecular weights of the samples of the κ-components examined were approximately 300,000[2]. Three alcohol fractions of κ-carrageenin were also studied[7]. The yields of these fractions were: at 35% alcohol, 3%; at 45% alcohol, 65% and in the supernatant 25%; unrecovered 7%. The samples of λ-carrageenin had molecular weights of 500,000–700,000[2]. One sample (CM-1-λ-3[2,7]) was fractionated with alcohol during the present work, the yields being: 34% at 33% alcohol (fraction 1); 33% at 38% alcohol (fraction 2); 10% at 41% alcohol (fraction 3) and 12% at 50% alcohol (fraction 4); unrecovered material 11%. Chromatographic analysis (after SMITH *et al.*[7]) showed that the first fraction, which has the highest molecular weight, consists mainly of D-galactose with only slight traces of xylose, glucose, L-galactose and an unidentified component. Later fractions contained increasing quantities of these other components.

* Issued as N.R.C. No. 3575.

References p. 205.

Infrared examination

A Perkin Elmer Model 21 Double beam Infrared Spectrophotometer was used with a sodium chloride prism. Specimens were prepared as Nujol mulls from precipitated materials which had been previously dried *in vacuo* over phosphorus pentoxide.

X-ray examination

(a) *Preparation of oriented specimens.* Unoriented samples of carrageenin give only broad diffuse diffraction halos. The most convenient and successful method of producing oriented specimens was found to be by stretching extruded fibres. Moistened films were difficult to orient by stretching, and rolling was unsatisfactory.

Fibres were formed by extruding a viscous aqueous solution of the material at 40° C through a drawn glass nozzle of about 1 mm bore into absolute ethanol or acetone at room temperature. The fibre was immediately withdrawn and, as it stiffened on drying in air, stretched to about twice its length. The optimum conditions for different samples differed only in minor details but, in general, fibres of κ-carrageenin were more difficult to produce than the others. Although the X-ray diffraction pictures from these fibres were inadequate for more than tentative structural analyses, attempts to improve the molecular orientation by further stretching were not successful. The maximum extension which could be produced in a fibre before breakage occurred was approximately 100%, whether this was got by stretching initially on drying or subsequently in a bath of 60% aqueous alcohol.

(b) *Density measurements.* The densities of fibres, determined by suspending them in a mixture of carbon tetrachloride and tetrabromoethane, were found to be 1.64 g/ml for κ-, 1.73 g/ml for λ-, and 1.66 g/ml for whole carrageenin. Since large portions of the fibres are evidently not well oriented, these estimates must be low for the ordered regions.

(c) *Moisture content.* Approximate estimates of the moisture contents of both precipitated materials and fibres were obtained by weighing samples after equilibrating for two weeks at 21° C and 50% R.H. and after subsequent drying at 21° C *in vacuo* over phosphorus pentoxide for 40 hours. Moisture contents of fibres of whole, κ- and λ-carrageenin were about 13%; the values for precipitated materials were about 3% higher. Although these results are in accord with those of other workers[7], they are probably too high for the well-ordered regions of the fibres.

(d) *Optical measurements.* The birefringence of fibres was estimated by revolving them in a holder on a microscope stage and measuring the phase retardation with a calibrated quartz wedge, and the thickness with an eyepiece micrometer. The results agreed within experimental error with estimates made by measuring the refractive indices of a few fibres parallel and perpendicular to their axes using immersion liquids. The fibres used for X-ray examination each had a positive birefringence of between 0.006 and 0.010; their thickness was usually 100–150 μ. The refractive indices of unoriented air-dried films, using immersion liquids, were 1.506 for κ- and 1.505 for λ-carrageenin.

(e) *X-ray apparatus.* A North American Philips microcamera with a Hilger and Watts microfocus X-ray set were used throughout the work. With the 100 μ collimator, a specimen-to-film distance of 1.5 cm and Ni-filtered CuKα radiation, the longest exposures required were 40–48 h at 40 KV and 400 μa. The camera was calibrated by dusting the fibres with finely ground sodium chloride. With the exception of some exposures on λ-carrageenin, the patterns were obtained at relative humidities below 50%.

INFRARED RESULTS AND DISCUSSION

Typical spectra for κ- and λ-carrageenin over the range 1350 cm^{-1} to 650 cm^{-1} are given in Fig. 1; the alcohol fractions of these components gave closely similar spectra. Although κ-carrageenin is known to contain an appreciable proportion of 3,6-anhydro-D-galactose residues[6,7] while λ-carrageenin does not, their spectra are reasonably similar. The most distinct difference between them is in the strengths of the peaks at 700 cm^{-1} and 925 cm^{-1}.

From the work of ORR[8], the pronounced absorption in the carrageenin spectra a- about 1240 cm^{-1} can be assigned with reasonable assurance to S = O stretching vit brations. The broad absorption band ascribed to C–O stretching and C–O–H bending modes occurs at about 1050 cm^{-1}. ORR (*loc. cit.*) has suggested that, in the spectra of sulphated polysaccharides, absorption due to stretching within the C–O–S system should occur in the region of 820–840 cm^{-1}, the actual position of this peak depending

References p. 205.

upon whether the sulphate group is axial or equatorial with respect to the plane of the pyranose ring. Since, on present evidence, all the sulphate groups in carrageenin are in the equatorial position, they should give rise to a single peak. This peak can probably be identified with the strong absorption at 840 cm^{-1}.

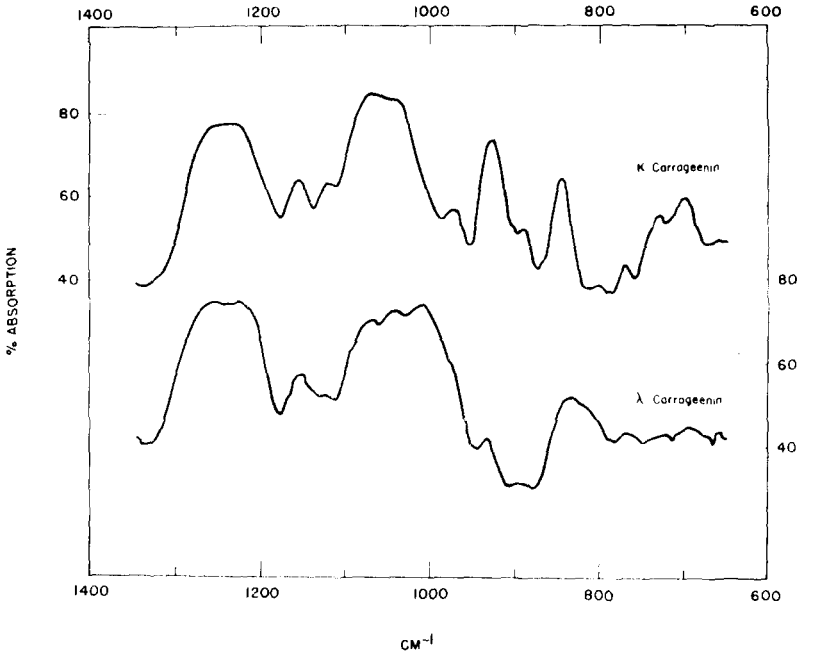

Fig. 1. Infrared spectra of κ- and λ-carrageenin.

X-RAY RESULTS

Fibres of κ-, λ- and whole carrageenin each gave a characteristic diffraction pattern (Figs. 2–5). Mean values for spacings calculated from the reflections using Bragg's equation are given in Tables I–III, columns 3. No significant differences could be detected between different preparations of the same material, between κ-carrageenin and its main (second) fraction, or between λ-carrageenin and its first fraction. Other fractions were not examined. The pattern obtained from a fibre of "recombined" whole carrageenin, *i.e.* κ- and λ-components mixed in solution in equal quantities, was identical with that of unfractionated material. There was no evidence that the three patterns represented different transition states arising from different degrees of orientation within the fibres.

With λ-carrageenin, which showed a strong equatorial reflection, exposures at different moisture contents were obtained by passing nitrogen or hydrogen at the desired humidity through the camera. The only change detected was an increase of about 0.2 A in the 5.8 A equatorial reflection at a relative humidity of 81%.

Whole carrageenin

With whole carrageenin, the reflections of spacing 10.5 A and 8.6 A suggest a fibre period of about 25 A. If the strong meridional reflection at 4.2 A is assumed to be the

References p. 205.

6th order reflection, this leads to a fairly accurate value of 25.2 A for the fibre period. The lack of distinct reflections other than on the meridian makes the choice of a unit cell unreliable, but reflections can be assigned satisfactorily assuming an orthorhombic cell with $a = 18.8$ A, b (fibre axis) $= 25.2$ A, and $c = 11.4$ A (Table I, columns 1 and 2).

TABLE I

X-RAY DATA FOR WHOLE CARRAGEENIN

hkl	$d_{calc.}$	$d_{obs.}$ (*Mean of 6 films*)	*Visually estimated intensity**
001	11.4 (assumed)	11.4	W
300	6.2	5.9	W
002	5.7		
202	4.8	4.4**	S
400	4.7		
401	4.3		
302	4.2		
120	10.5	10.5	S
221	6.2	*ca.* 6.0	VW
222	4.5	4.4	S
420	4.4		
030	8.4	8.6	MW
131	6.3	*ca.* 6.0	VW
230	6.2		
050	5.0	4.9	VS
150	4.9		
060	4.2 (assumed)	4.2	VS
070	3.6	3.5	M
080	3.2		
090	2.8	2.8	W
0, 10, 0	2.5	2.3	VW
2, 10, 0	2.4		

* S = strong; M = medium; W = weak; V = very.

** Broad region of scatter where individual reflections are not resolved.

Figs. 2–5. Representative X-ray fibre diagrams from carrageenin. Fibre axis vertical.

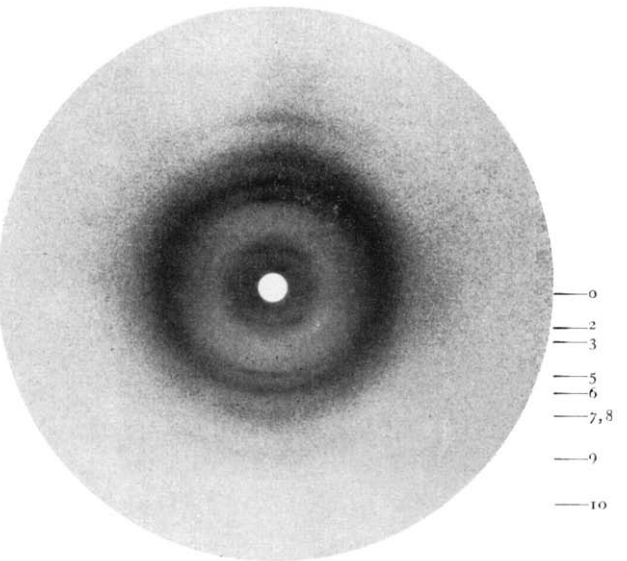

Fig. 2. Whole carrageenin.

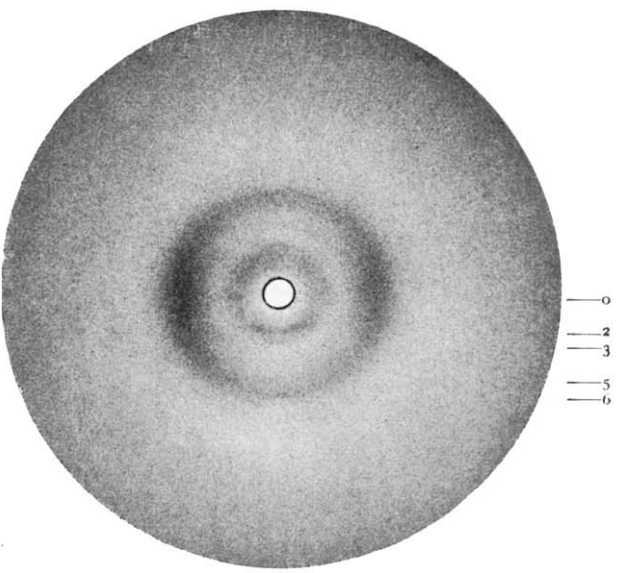

Fig. 3. Whole carrageenin to show reflections on second and third layer lines.

κ-Carrageenin

κ-Carrageenin does not give as distinct a pattern as whole carrageenin but the general features show similarities. In particular the fibre periods appear to be identical and the agreement within experimental error between the (120) reflections in whole and κ-carrageenin suggests that the

References p. 205.

a axis of the unit cell is the same. The reflections are not clear enough to lead to a reliable value for the *c* axis, but reasonable agreement is obtained if indices are assigned to the reflections on the basis of the unit cell for whole carrageenin (Table II, columns 1 and 2). The diagram from κ-carrageenin differs from that of whole carrageenin chiefly in the absence or extreme weakness of the 4.9 A meridional reflection; also the 8.4 A appears to be weaker and has not been clearly resolved.

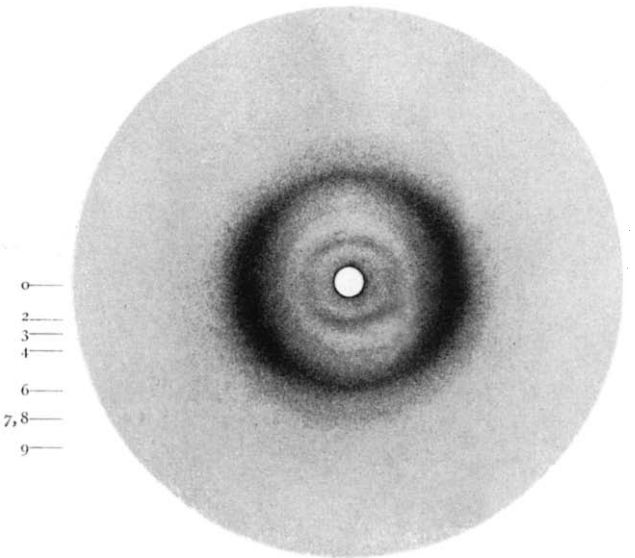

Fig. 4. κ-Carrageenin.

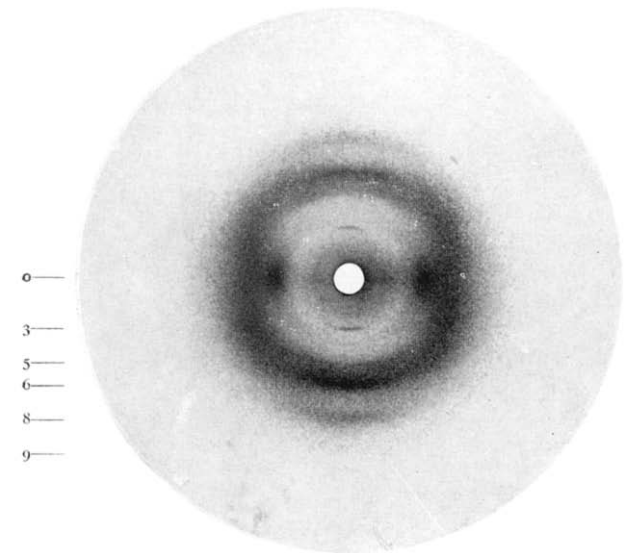

Fig. 5. λ-Carrageenin.

TABLE II

X-RAY DATA FOR κ-CARRAGEENIN

hkl	$d_{calc.}$	$d_{obs.}$ (*Mean of 5 films*)	*Visually estimated intensity**
001	11.4 (assumed)	*ca.* 11.0	VW
300	6.2	*ca.* 5.9	VW
002	5.7		
202	4.8	4.4**	VS
400	4.7		
401	4.3		
302	4.2		
120	10.5	10.6	S
221	6.2	6.2	W
030	8.4	8.6	W
230	6.3	6.2	W
131	6.2		
040	6.3	6.2	W
140	6.0		
060	4.2	4.3	VS
070	3.6	3.4	M
080	3.2		
090	2.8	2.8	W

* See Table I. ** See Table I.

TABLE III

X-RAY DATA FOR λ-CARRAGEENIN

hkl	$d_{calc.}$	$d_{obs.}$ (*Mean of 9 films*)	*Visually estimated intensity**
100	11.2 (assumed)	11.2	MW
001	5.9 (assumed)	5.8**	VS
200, 101	5.6		
201	4.4 (assumed)	4.4**	S
030	8.4	8.4	M
231	3.9	4.0**	S
050	5.0	5.0**	M
060	4.2	4.1	VS
080	3.2	3.3	M
090	2.8	2.8	W

* See Table I. ** See Table I.

References p. 205.

λ-Carrageenin

The diagram from λ-carrageenin suggests a fibre period identical with those for whole and κ-carrageenin. However, the λ-carrageenin diagram differs from the others in having a strong equatorial reflection at 5.8 A and a clearly defined meridional reflection at 8.4 A. In addition there are no distinguishable reflections on the second layer line, and the 5.0 A meridional reflection is weaker and more blurred than in whole carrageenin. The strong equatorial reflections centred at 5.8 A and about 4.4 A appear to be multiple. By assuming the interplanar spacing of the (001) planes to be 5.9 A and giving the 4.4 A reflection the indices (201), a satisfactory monoclinic unit cell is obtained with $a = 11.3$ A, b (fibre axis) $= 25.2$ A, $c = 6.0$ A, and $\beta = 81°$. Assignments and calculated spacings for this cell are given in Table III, columns 1 and 2.

DISCUSSION OF X-RAY RESULTS

λ-Carrageenin

For reasons which will become evident, this component will be considered first. SMITH *et al.*[7] have shown that λ-carrageenin is largely a sulphated poly-D-galactan and, from the earlier work of PERCIVAL and co-workers[1,3], it is assumed that the residues are linked α-1,3- with the sulphate group on carbon 4. The small quantities of L-galactose, xylose and glucose in λ-carrageenin can safely be disregarded in the present discussion since the first fraction of λ-carrageenin from which these other sugar residues are absent also gives the usual λ-carrageenin diagram.

The strength and clarity of the 8.4 A meridional reflection with the absence of reflections on other low-order layer lines suggests that the 25.2 A period is composed in λ-carrageenin of three closely similar subunits. The presence of the 5.0 A and 3.3 A reflections, however, prevents these subunits from being related by a three-fold screw axis.

The elastic properties of the fibres suggest that the molecular chains are fully extended and from measurements on molecular models (Fig. 6) it is found that the 8.4 A fibre subperiod can be represented by the chain length of two galactose residues joined by an α-1,3-linkage. This interpretation supports the earlier suggestion by BUCHANAN *et al.*[9]. In the present discussion, the residues are assumed to be in the chair form of the pyranose ring. For this, two configurations are possible, designated C1 and 1C by REEVES[10]. With the α-1,3-linkage, however, these two configurations lead to identical fibre periods so that it is impossible to distinguish between them. With

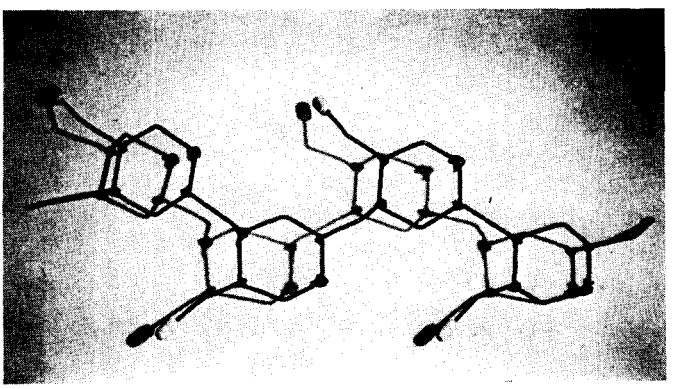

Fig. 6. Model of λ-carrageenin composed of sulphated D-galactose residues in the 1C configuration linked α-1,3-. Here and in Fig. 8, the side arms represent ester sulphate groups.

References p. 205.

the β-1,3-linkage, the fibre period is *ca.* 9.0 A for C1 residues but is stereochemically improbable for 1C.

From the strength of the 8.4 A reflection, the two galactose residues within this period evidently form a distinct "galactobiose" unit and cannot be equivalent crystallographically, although at present there is no satisfactory evidence to account for any chemical differences between them. This result is reminiscent of the unit of cellobiose in cellulose (*e.g.* HERMANS[11]), and of chitobiose in chitin[12*]. However, the simple model shown in Fig. 6 is inadequate to explain the results of periodate oxidation of λ-carrageenin. SMITH *et al.*[7] suggest that some sulphate groups may be distributed unevenly among the galactose residues and that occasional side residues may be present. It seems likely that these features account for the 25.2 A periodicity in λ-carrageenin although the chemical data are insufficient for a more detailed interpretation.

From the observed density and water content of λ-carrageenin, the number of units of six sulphated galactose residues each within the proposed unit cell is calculated to be one. In this work, the data are insufficient to permit the determination of space groups, but some idea of the molecular packing can be got from the relative intensities of reflections. In arranging the λ-carrageenin molecule within its unit cell, account must be taken of the strength of the (101) and (201) reflections, the comparative weakness of the (100) reflection, and the extreme weakness or complete absence of the (101) reflection. The form of a molecule composed of pyranose rings joined by α-1,3- linkages approaches that of a flat ribbon and the above conditions are satisfied if the planes of the molecules closely approximate to the (101) planes as in Fig. 7.

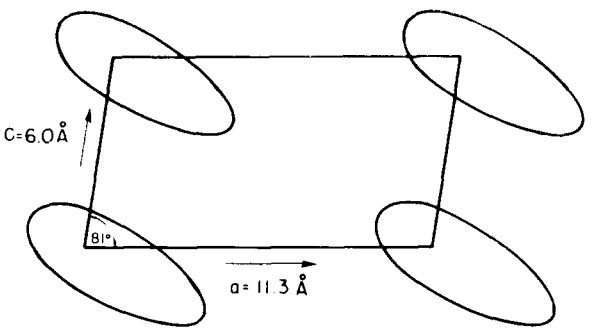

Fig. 7. Packing arrangement of λ-carrageenin molecules in a–c plane.

The proposed structure does not explain the intensity of the 5.0 A and 3.3 A meridional reflections. If the present cell were doubled, alternate molecules could be displaced relative to one another along the fibre axis. However, doubling the c axis is unsatisfactory since the spacings of the resulting (051) and (031) planes would be incorrect. Doubling the a axis would be satisfactory but is not justified by the X-ray evidence.

κ-Carrageenin

Chemical analyses[6,7] have shown that κ-carrageenin contains both sulphated D-galactose and 3,6-anhydro-D-galactose residues. Their ratio differs slightly between the three alcohol fractions, but the average is about 1.4:1[7]. The mechanical properties of κ-carrageenin fibres suggest that the molecular chains are fully extended. In the 25.2 A fibre period, therefore, the sulphated residues, assumed to be in α-1,3-linkage,

* In the Stuart model for cellulose (see HERMANS[11]), successive glucose residues are made crystallographically distinct by alternating the direction of the hydroxyl groups on carbons 2 and 6. A similar explanation may apply here with the sulphate groups except that, as these have considerably greater scattering power, the distinction between successive galactose residues would be demonstrated much more clearly.

References p. 205.

must have chain lengths of 4.2 A as shown by the results on λ-carrageenin. The anhydro-galactose residues probably have a closely similar chain length; the strength and clarity of the 4.2 A reflection from whole and κ-carrageenin support this suggestion and, moreover, no integral number of pyranose rings of widely different chain length could be accommodated. The six residues within the 25.2 A period of κ-carrageenin must therefore be sulphated galactose and anhydro-galactose in the ratio of either 1:1 or 2:1. The experimental value of 1.4:1 may be accounted for by branching, evidence for which in unfractionated carrageenin has been presented by JOHNSTON AND PERCIVAL[3]. Periodate oxidation of κ-carrageenin has shown that terminal residues, if present, are not sulphated galactose; however, terminal anhydro-galactose residues are permissible[7]. The residue ratio in the main chain therefore is probably 2:1. If in each 25.2 A period one anhydro-galactose residue is attached to the main chain through carbon 6 of a sulphated galactose residue, the ratio of the two residues for the whole molecule becomes 4:3, which is close to the experimental value.

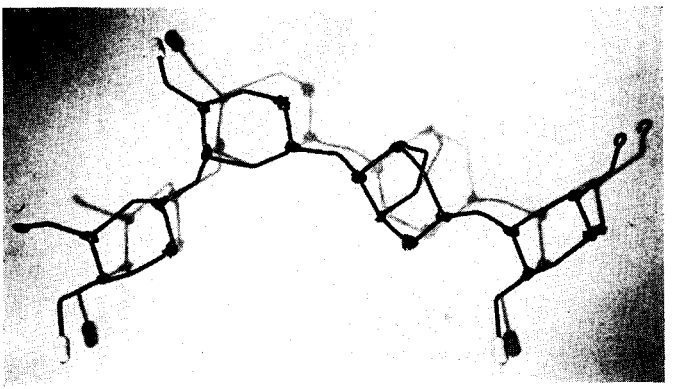

Fig. 8. Model of the main chain of κ-carrageenin composed of sulphated D-galactose residues linked α-1,3- and 3,6-anhydro-D-galactose residues linked β-1,4- in the ratio 2:1. Both types of residue are in the 1C configuration.

The chain lengths of 3,6-anhydro-D-galactose residues in α-1,4-linkage would be considerably longer than the 4.2 A required above (*cf.* the β-glucose residues in cellulose[11]), although the anhydro ring may shorten the residues by straining the pyranose ring. However, a model of 3,6-anhydro-D-galactose in the 1C chair form shows comparatively little strain of the valence bonds and angles, while in the C1 form the strain in forming the anhydro ring would be so excessive as to be improbable. A more satisfactory explanation is obtained by assuming a β-linkage which for an unstrained residue in the 1C form leads to a length of 4.35 A (*cf.* α-glucose residues in starch[13]). This distance may easily be reduced slightly by the strain from the anhydro ring.

The main chain of the κ-carrageenin molecule can therefore be represented by a succession of pairs of sulphated D-galactose residues in α-1,3-linkage interspersed with single 3,6-anhydro-D-galactose residues in β-1,4-linkage. A model of this structure is shown in Fig. 8. 3,6-anhydro-D-galactose residues are attached to sulphated galactose residues in this backbone at 25.2 A intervals so that the fibre repeat unit of the κ-carrageenin molecule consists of four sulphated D-galactose and three 3,6-anhydro-D-galactose residues.

Because of the diffuseness of the κ-carrageenin diagrams, molecular packing will be discussed only in relation to whole carrageenin.

A unique feature of κ-carrageenin is its sensitivity to potassium, ammonium,

References p. 205.

rubidium and caesium ions and insensitivity to lithium and sodium. It is interesting, although on present structural evidence perhaps premature, to attempt an explanation of this behaviour in terms of the proposed model for κ-carrageenin.

The kind of ions to which κ-carrageenin is sensitive suggests that the sensitivity depends upon the diameter of the hydrated cation[2], in particular that it must be smaller than a certain value, and that the smaller ions are able to pack κ-carrageenin molecules sufficiently closely to maintain aggregation and cause precipitation. A possible packing arrangement is shown in Fig. 9 in which two κ-carrageenin molecules are held closely together by electrostatic forces between their nearest sulphate groups and the univalent cations. Molecules above and below the plane of these two molecules could be displaced as shown, so that each sulphate group would be effectively surrounded by cations. In this regard the essential difference between κ- and λ-carrageenin molecules is that in the former, with the longer repeat period, successive sulphate groups on alternate neighbouring molecules are 6.3 A apart, whereas in the latter they would be only 4.2 A apart. Evidently the latter distance must be too small to allow univalent cations of any size to build up a structure of the type envisaged.

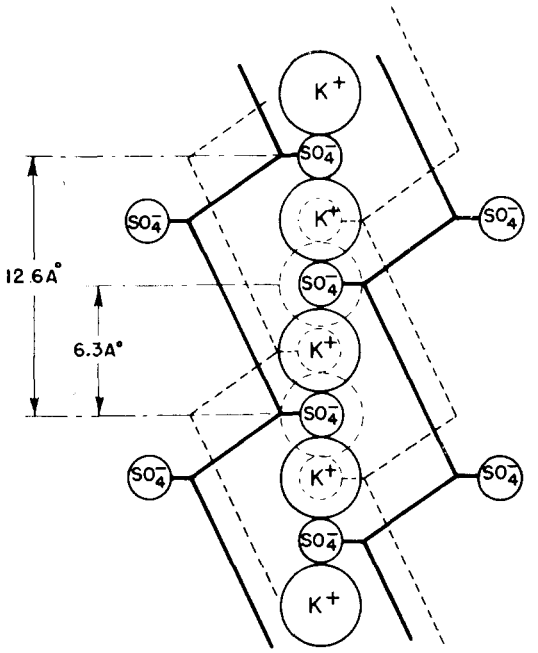

Fig. 9. Possible mode of aggregation of κ-carrageenin molecules in solution with hydrated univalent cations.

From measurements on space-filling models the diameter of the hydrated ion required in Fig. 9 is about 4 A. Values reported by different authors for the size of hydrated cations vary considerably (for summary see KACHMAR AND BOYER[14]), but all agree that K^+, NH_4^+, Rb^+ and Cs^+ are closely similar while Na^+ and Li^+ are respectively about 1.6 and 2 times greater. The size requirement here agrees most closely with the estimates of GORIN[15] and MOELWYN-HUGHES[16], who give the diameter of the hydrated K^+ ion as 2.5–4.0 A.

Excess potassium chloride is required to precipitate κ-carrageenin[5]. The explanation may be that, as the molecules are excessively long and highly charged, the sulphate groups must be effectively neutralized before appreciable portions of the molecules can approach at all closely. The lower potassium chloride concentration required in the presence of sodium chloride[5] may be explained in the same way.

The packing arrangement suggested in Fig. 9 requires an ordered crystalline lattice that would exist only in the hydrated state and would be destroyed on drying. However, it may be possible to detect aggregation by light scattering measurements at concentrations of potassium ions below the level at which precipitation occurs.

Whole carrageenin

The whole carrageenin samples studied consisted of κ- and λ-components in nearly

References p. 205.

equal amounts. The unit cell, therefore, must contain equal numbers of κ- and λ-carrageenin chains of the types discussed above; that is, it must contain a multiple of ten sulphated galactose and three anhydro-galactose residues. The number of such structural units within the proposed cell is calculated to be about 1.6. Because of the possibly low density and high moisture estimates used in the calculation however, this unit cell probably contains two κ- and two λ-carrageenin chains.

As previously stated, space groups cannot be determined with any certainty from the present limited X-ray data and, although the unit cell was described earlier as orthorhombic, a reasonably satisfactory packing arrangement giving a monoclinic cell with $\beta = 90°$ can be obtained as shown in Fig. 10. In this, the absence or weakness of the (100), (200) and (001) reflections would be accounted for by the fairly close similarity of κ- and λ-carrageenin chains. Owing to the lack of resolution within the equatorial reflections at about 4.5 A it is difficult to decide the orientation of the planes of the molecules. From the weakness of the (002) reflection, however, the orientation must be approximately parallel to the (100) planes. The side residues of the κ-carrageenin molecules could be accommodated in this packing arrangement if they protruded along the a axis, *i.e.* perpendicular to the general plane of the backbone residues. The strong (120) reflection probably arises from the κ-carrageenin molecules alone since it persists in the κ-carrageenin diagram as well. This reflection may be due to a relative displacement of $b/4$ of alternate κ-carrageenin chains along the a axis; the side residues may also contribute to it.

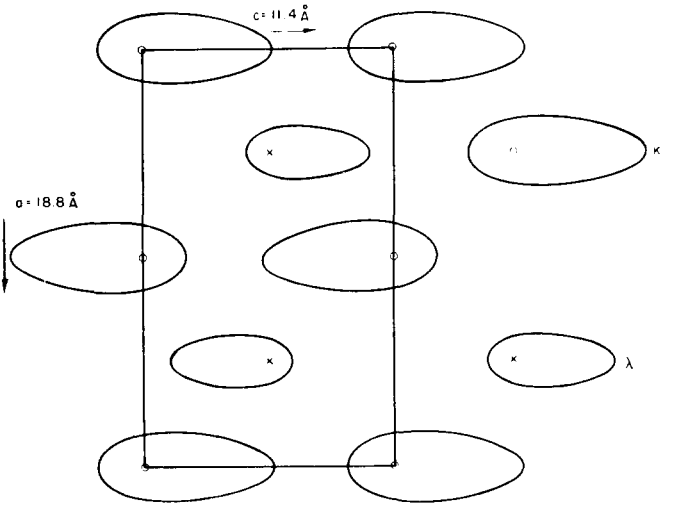

Fig. 10. Packing arrangement of molecules in a-c plane in whole carrageenin.

The strong 4.9 A meridional reflection may be due to the packing of the λ-carrageenin molecules, although it is considerably clearer and stronger with whole than with λ-carrageenin. If the reflection arose from two arcs off the meridian and were given the indices (150), it would then represent a displacement of the λ-carrageenin molecules relative either to one another or to the κ-carrageenin chains.

Assuming the unit cell for κ- to be the same as for whole carrageenin, the number of κ-carrageenin chain units of four sulphated galactose and three anhydro-galactose residues each within this cell is calculated to be 3.2, *i.e.* four molecules traverse the cell. This result and the general similarity of the X-ray diagrams suggest that the packing in κ- is similar to that in whole carrageenin with κ-carrageenin molecules replacing the λ-. The condition giving rise to the (150) reflection, however, no longer obtains.

CONCLUSION

Comparison of the X-ray diffraction patterns of κ-, λ- and whole carrageenin shows

References p. 205.

that the pattern from whole carrageenin does not represent the sum of those from the κ- and λ-components separately. In whole carrageenin, therefore, the two types of molecules must exist in a distinct and definite structural relationship with respect to one another and cannot occur as large separate aggregates. Separation of κ- and λ-components with subsequent recombination does not appear to destroy irreversibly any major structural features in whole carrageenin.

Despite known chemical differences between κ- and λ-carrageenin[6,7], their infrared spectra are fairly similar. The X-ray diffraction patterns from these two components, however, confirm chemical analyses in establishing the existence of structural differences between them. The fibre period of 25.2 A can be accounted for in the proposed structure for κ-carrageenin and in λ-carrageenin, although more definite and detailed structural analyses must await further chemical studies.

ACKNOWLEDGEMENTS

The author is grateful to Dr. D. B. SMITH for providing most of the samples and thanks him and Dr. A. S. PERLIN for valuable discussions at all stages of the work. Thanks are due to Drs. W. H. COOK, W. H. BARNES and E. O. HUGHES for criticizing the manuscript. It is a pleasure to thank Mr. F. ROLLIN for able technical assistance in the preparation of specimens and for running the infrared spectra.

SUMMARY

X-ray diffraction patterns have been obtained from stretched fibres of whole carrageenin and its κ- and λ-components. The results establish structural differences between the two components, but the proposed fibre period in all three materials is 25.2 A.

The κ-carrageenin molecule is probably branched. Along the main chain, the fibre period appears to contain two trisaccharide units each comprising two sulphated D-galactose residues linked α-1,3- and one 3,6-anhydro-D-galactose residue linked β-1,4-, while within each 25.2 A period a single side residue of 3,6-anhydro-D-galactose appears to be attached to the main chain through carbon 6 of a sulphated D-galactose residue.

In λ-carrageenin the fibre period may represent three disaccharide units the majority of which are composed of two sulphated D-galactose residues linked α-1,3-. The long fibre period could be accounted for by a variation in the number of sulphate groups attached to the galactose residues or by the presence of side residues.

λ-Carrageenin appears to have a monoclinic unit cell with $a = 11.3$ A; b (fibre axis) $= 25.2$ A; $c = 6.0$ A and $\beta = 81°$. This cell is traversed by one λ-carrageenin molecule. The unit cells for whole and κ-carrageenin are identical, and it is suggested that they are probably monoclinic with $\beta = 90°$ and axes $a = 18.8$ A; b (fibre axis) $= 25.2$ A and $c = 11.4$ A. In κ-carrageenin, four molecules traverse such a unit cell, while whole carrageenin it is traversed by two κ- and two λ-carrageenin molecules which alternate in sheets parallel to (100). The infrared spectra of the κ- and λ-components are similar. The potassium sensitivity of κ-carrageenin is explained in terms of its proposed structure.

RÉSUMÉ

Les images de diffraction des rayons X par des fibres étendues de carragénine totale et de ses constituants κ et λ ont été obtenues. Les résultats indiquent des différences structurales entre les deux constituants, mais la période proposée pour la fibre est, pour les trois produits, de 25.2 A.

La molécule de κ-carragénine est probablement ramifiée. Le long de la chaine principale, la période de la fibre contient deux unités trisaccharidiques, comprenant deux résidus D-galactose sulfatés liés en α-1,3, et un résidu 3,6-anhydro-D-galactose lié en β-1,4-, tandis que, à l'intérieur de chaque période de 25.2 A, un seul résidu latéral de 3,6-anhydro-D-galactose est relié à la chaîne principale par l'intermédiaire du carbone 6 d'un résidu D-galactose sulfaté.

Dans la λ-carragénine, la période de la fibre peut représenter trois unités disaccharidiques, la majorité desquelles sont composées de deux résidus D-galactose sulfates liés en α-1,3-. La période

References p. 205.

longue de la fibre peut être rapportée à une variation dans le nombre des groupes sulfate liés au résidus galactose ou à la présence de résidus latéraux.

La λ-carragénine possède une unité monoclinique pour laquelle $a = 11.3$ A; b (axe de la fibre) $= 25.2$ A; $c = 6.0$ A et $\beta = 81°$. Cette unité est traversée par une molécule de λ-carragénine. Les unités de la carragénine totale et de la κ-carragénine sont identiques et l'on peut supposer qu'elles sont monocliniques avec $\beta = 90°$ et des axes $a = 18.8$ A; b (axe de la fibre) $= 25.2$ A et $c = 11.4$ A. Dans la κ-carragénine, quatre molécules traversent une telle unité, tandis que dans la carragénine totale, elle est traversée par deux molécules de κ et deux molécules de λ-carragénine qui alternent en feuillets parallèles à (100). Les spectres infra-rouges des constituants κ et λ sont semblables. La sensibilité au potassium de la κ-carragénine est expliquée à l'aide de la structure proposée.

ZUSAMMENFASSUNG

X-Strahlendiffraktionsbestimmungen wurden mit gedehnten Fasern von ganzem, sowie in seine κ- und λ-Komponenten aufgespaltenem Carrageenin durchgeführt. Die Ergebnisse beweisen, dass zwischen beiden Komponenten strukturelle Unterschiede bestehen, dass aber die gefundene Faserperiodizität der drei Substanzen in allen Fällen 25.2 A ist.

Das κ-Carrageenin-Molekül ist wahrscheinlich verzweigt. In der Hauptkette scheint eine einzelne Faserperiode aus zwei Trisaccharideinheiten zu bestehen, deren jede ihrerseits aus zwei sulfatierten D-Galaktoseresten in α-1,3-Bindung und einem 3,6-anhydro-D-Galaktoserest in β-1-4-Bindung besteht, wobei andererseits von jeder 25.2 A — Periode der Hauptkette ein einzelner 3,6-anhydro-D-Galaktoserest abzweigt. Dieser Galaktoserest scheint über das 6-Kohlenstoffatom eines sulfatierten D-Galaktoserests mit der Hauptkette verbunden zu sein.

Im λ-Carrageenin kann die Faserperiode aus drei Disaccharideinheiten bestehen von denen die meisten je zwei sulfatierte D-Galactosereste in α-1,3-Bindung aufweisen, wobei die Länge der Faserperiode durch eine Änderung in der Zahl der an die Galaktosereste gebundenen Sulfatgruppen, oder durch Seitenketten erklärt werden kann.

λ-Carrageenin scheint eine monoklinische Einheitszelle mit $a = 11.3$ A, $b = 25.2$ A (Faserachse), $c = 6.0$ A und $\beta = 81°$ zu besitzen. Diese Zelle wird nur durch ein einziges λ-Carrageeninmolekül durchzogen.

Die Einheitszelle für ganzes und κ-Carrageenin ist dieselbe, es wird vorgeschlagen, dass sie wahrscheinlich monoklinisch, mit einem β von 90° und Achsen von $a = 18.8$ A, b (Fasernachse) $= 25.2$ A und $c = 11.4$ A ist. Eine solche Zelle wird in κ-Carrageenin von vier Molekülen durchzogen, während sie in ganzem Carrageenin von zwei κ- und zwei λ-Carrageeninmolekülen, welche in zu (100) parallelen Schichten miteinander abwechseln, durchzogen wird.

Das infrarote Spektrum der κ- und λ-Komponente ist dasselbe.

Die Kaliumempfindlichkeit des κ-Carrageenins wird an Hand der vorgeschlagenen Struktur erklärt.

REFERENCES

1 For review see E. G. V. PERCIVAL, *Quarterly Reviews, Chem. Soc.*, 3 (1949) 369.
2 D. B. SMITH, W. H. COOK AND J. L. NEAL, *Arch. Biochem. Biophys.*, 53 (1954) 192.
3 R. JOHNSTON AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 1944.
4 W. H. COOK, R. C. ROSE AND J. R. COLVIN, *Biochim. Biophys. Acta*, 8 (1952) 595.
5 D. B. SMITH AND W. H. COOK, *Arch. Biochem. Biophys.*, 45 (1953) 232.
6 A. N. O'NEILL, presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society, New York, N.Y. (1954).
7 D. B. SMITH, A. N. O'NEILL AND A. S. PERLIN (to be published).
8 S. F. D. ORR, *Biochim. Biophys. Acta*, 14 (1954) 173.
9 J. BUCHANAN, E. E. PERCIVAL AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1943) 51.
10 R. E. REEVES, *J. Am. Chem. Soc.*, 72 (1950) 1499.
11 P. H. HERMANS, *Physics and Chemistry of Cellulose Fibres*, Elsevier Pub. Co., New York, 1949.
12 K. H. MEYER AND G. W. PANKOW, *Helv. Chim. Acta*, 18 (1935) 589.
13 D. R. KREGER, *Biochim. Biophys. Acta*, 6 (1951) 406.
14 J. F. KACHMAR AND P. D. BOYER, *J. Biol. Chem.*, 200 (1953) 669.
15 M. H. GORIN, *J. Chem. Phys.*, 7 (1939) 405.
16 E. A. MOELWYN-HUGHES, *Physical Chemistry*, Cambridge, 1940, p. 39–49.

Received December 15th, 1954