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**EFFECT OF POLYMERIZATION DEGREE OF OLIGOGALACTURONATES AND D-GALACTURONANS ON THEIR CIRCULAR DICHROIC SPECTRA**

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The CD spectra of aqueous solutions of homopolymeric sodium oligogalacturonates and D-galacturonans of polymerization degree  $n = 1-64$ , as well as lower calcium oligogalacturonates ( $n = 1-5$ ) were measured. Chiroptic properties were correlated with the polymerization degree in terms of optical superposition of monomeric unit increments. Interpretation of obtained data, respecting further physicochemical properties entitles to conclude that the conformation of macromolecules of D-galacturonan in solution is close to helical structure.

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Circular dichroism was chosen to elucidate interactions of cations with the appropriate polyanions<sup>1</sup> as a part of continuing program on physicochemical properties of glycuronans in solutions. This method does not inform on interactions of the chromophoric system only, but reflects also the conformational arrangement of molecules. The conformation of a pectin molecule in solid state was investigated in detail by means of X-ray diffraction analysis. The molecule of pectic acid and its sodium salt was characterized by a threefold screw symmetry<sup>2</sup>. The molecule of calcium pectate<sup>3</sup> and O-acetyl derivatives of pectic acid<sup>4</sup> displays, on the other hand, a twofold screw symmetry. The conformation of pectin macromolecule in aqueous solutions has been relatively little examined as yet. Viscometric measurements<sup>5-7,21</sup> lead to a conclusion that the molecule of pectin in solution, like molecules of alginate, can be best characterized as very extended random coils. Theoretic calculations indicate the probable conformations of these macromolecules<sup>8-10</sup>.

The aim of this paper is to contribute to the stereochemistry of the pectin molecule in aqueous solution making use of circular dichroism of defined model substances; these were homopolymeric oligogalacturonates and D-galacturonans. Macromolecules of these substances are no more flexible to the contrary of pectin molecule, where a certain flexibility can be considered. This flexibility is due to some L-rhamnose units incorporated in the linear chain of D-galacturonic acid units<sup>8,10</sup>. It has been found<sup>11</sup> that chiroptic properties of several acid polysaccharides are close to those of monomeric units in the sense of linear combination of these components. Sodium pectate is characterized by a single chiroptic band of a positive sign like its monomer sodium D-galacturonate. The spectra, however, differ in the intensity of the band and position

of the maximum. It has not been analysed what is responsible for the change in the spectrum and how the conformation pattern of macromolecules is reflected.

## EXPERIMENTAL

D-Galacturonic acid (monohydrate) puriss. was a preparation of Fluka (Buchs, Switzerland). Oligogalacturonates and sodium D-galacturonans were prepared by a partial acid hydrolysis of purified pectic acid followed by rechromatography and desalting on Sephadex columns. Sodium oligogalacturonates of degree of polymerization  $n = 2$  to 5, prepared and characterized as published earlier<sup>12</sup>, were homopolymeric and homodisperse. The oligomer ( $n = 8$ ) of the same quality was supplied by Dr K. Heinrichová of this Institute.

D-Galacturonans of various polymerization degree ( $n = 15$  to 64) were preparations used in the preceding paper<sup>13</sup>. These substances were virtually homopolymeric and contained more than 99% of D-galacturonan in the dry substance; of neutral saccharides only D-galactose units were present in an amount not exceeding 1%. The presence of L-rhamnose in preparations was not proved. Preparations showed a very narrow distribution of molecular weights within  $\pm 3$  to  $\pm 5\%$ .

Pectic acid, prepared by an alkaline deesterification of citrus pectin contained 90% of D-galacturonan and 10% of neutral saccharides of which L-rhamnose represented less than 0.3%. Its average degree of polymerization  $n = 91$  was estimated viscometrically<sup>5</sup>. For a more detailed characterization of preparations and analytical methods see<sup>13</sup>. Sodium pectate was prepared by neutralization with sodium hydroxide.

Solutions prepared from starting samples (concentration 3–4 mequiv.[COONa]/l) were percolated over a Dowex 50 WX2 ( $H^+$ -form) column. Resulting solutions of uronic acids were titrated with the corresponding hydroxide exactly to the point of equivalence (pH  $\sim 7.2$ ). Used were solutions 0.05M-NaOH (carbonate free) and 0.021M-Ca(OH)<sub>2</sub> (saturated calcium hydroxide solution). The concentration of solutions for chiroptical measurements was adjusted to 2.00 mequiv. [COOM]/l ( $M = Na$  or  $Ca_{0.5}$ ). The redistilled water was CO<sub>2</sub>-free.

The CD spectra were recorded with a Roussel-Jouan Dichrograph, model 185, at room temperature in 0.2 and 0.5 cm-cells.

## RESULTS

Chiroptical parameters were obtained of sodium oligogalacturonates and sodium D-galacturonans of polymerization degree  $n = 1$  to 64 and calcium oligogalacturonates of  $n = 1$  to 5 (Table I). Calcium oligogalacturonates were studied in the low-molecular region only, where molecular disperse solutions are involved. In the region of higher polymerization degree ( $n > 10$ ) the aggregation of macromolecules and formation of gels<sup>13</sup> take place. On the other hand, the complete series of sodium oligogalacturonates and D-galacturonans could be studied by CD.

The substances show a simple positive band belonging to a  $n \rightarrow \pi^*$  electronic transition of the carboxyl group. The intensity of the band associated with one chromophoric group  $[\theta]_n$  (molar residual ellipticity) rises with the increase of the size of the molecule and at higher polymerization degrees of Na salts ( $n > 15$ ) it approaches asymptotically the constant value (Fig. 1). The dependence  $[\theta]_n$  on  $n$  shows that the extension of the chain length does not lead to formation of new structures, which depends on co-operative intramolecular interactions. It could be,

therefore, assumed that the principle of optical superposition of contributions of monomeric units to the total optical activity holds. Optical superposition, *i.e.* additivity of optical rotation in macromolecules was observed also with other polysaccharides and their derivatives<sup>14</sup>. This phenomenon was first described by Freudenberg<sup>15</sup>.

TABLE I

Circular Dichroism of Solutions of Oligogalacturonates and D-Galacturonans (2.00 mequiv. [COOM]/l; M = Na, Ca<sub>0.5</sub>)

<i>n</i>	$[\theta]_n/n \cdot 10^{-3}$		$\lambda_n$ , nm	
	Na	Ca	Na	Ca
1	1.32	1.39	209.5	209.5
2	1.85	1.86	206.0	206.0
3	2.11	2.29	205.0	203.0
4	2.31	2.35	204.0	201.0
5	2.48	2.48	203.5	200.0
8	2.54		203.0	
15	2.64		202.5	
21	2.77		202.0	
30	2.87		201.5	
64	2.80		202.5	
91 <sup>a</sup>	2.80		202.0	

<sup>a</sup> Sodium pectate;  $[\theta]$  (degree cm<sup>2</sup> dmol<sup>-1</sup>).

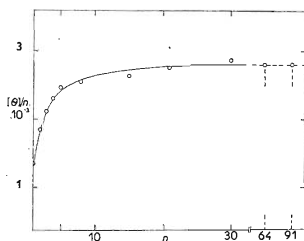


FIG. 1

Relationship between the Molar Residual Ellipticity  $[\theta]_n/n$  and the Polymerization Degree *n* of Sodium Oligogalacturonates and D-Galacturonans

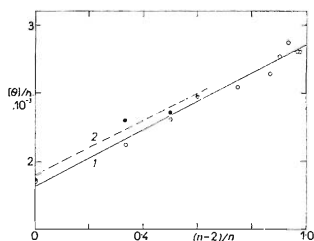


FIG. 2

Plot of  $[\theta]_n/n = f((n-2)/n)$  Sodium Oligogalacturonates and D-Galacturonans 1 and Calcium Oligogalacturonates 2

The mathematic expression

$$[\theta]_n = [\theta]_2 + (n - 2)[\theta] \quad (1)$$

for region  $n \geq 2$  indicates that optical activity of a  $n$ -membered polymer  $[\theta]_n$  can be expressed as a sum of activities of both terminal units  $[\theta]_2$  and central units  $[\theta]$ . After rearrangement

$$[\theta]_n/n = [\theta]_2/2 + ([\theta] - [\theta]_2/2)(n - 2)/n. \quad (2)$$

The relationship  $[\theta]_n/n$  versus  $(n - 2)/n$  for sodium oligogalacturonates and D-galacturonans and calcium oligogalacturonates is of linear character (Fig. 2); deviations from the measured values are very close to experimental error. According to equation (2) the section on the ordinate represents the mean value of optical activities of both terminal units, the line slope expresses the difference between molar residual ellipticity of the central unit and the mean value of terminal units. The values of these parameters obtained from the graphic relationship by the method of least squares are  $[\theta]_2/2 = 1825$ ;  $[\theta] - [\theta]_2/2 = 1019$  for Na salts and 1900 and 990, respectively for Ca salts. Correlation coefficients are 0.990 and 0.971 for the dependence of Na and Ca salts, respectively.

As it follows from the virtually consonant course of lines drawn in Fig. 2 and values  $[\theta]_n/n$  (Table I), sodium and calcium oligogalacturonates reveal the same chiral properties. From the value of the line slope it is evident that the rotation strength representing the inner unit is higher (more positive) than that of the terminal unit. This increment is the result of interaction between monomeric units, or possibly of the change of intramolecular interactions within the monomeric unit.

The measurement of the CD spectra with Roussel-Jouan Dichrograph enabled, in contrast to the preceding paper<sup>1</sup>, a more exact examination of the relation between the change of wavelength of maximum ( $\lambda_n$ ) and the degree of polymerization. The wavelength associated with the dichroic band of  $n \rightarrow \pi^*$  transition was hypsochromically shifted with the extension of the D-galacturonan chain length. Calcium oligogalacturonates were found to have this shift more pronounced than sodium oligogalacturonates (Table I). The relationship for sodium salts (Fig. 3) is analogous to that of intensity bands at value  $n$  (Fig. 1). Therefore, the relation of wavelength  $\lambda_n$  versus  $(n - 1)/n$  (Fig. 4) was also investigated. A linear dependence was found (Na salts - curve 1); it can be expressed by equation

$$\lambda_n = \lambda_1 + \Delta\lambda(n - 1)/n. \quad (3)$$

The section on the ordinate  $\lambda_1$  is identical with the wavelength of maximum of the band belonging to the monomer. The line slope  $\Delta\lambda$  represents the maximum possible

difference of wavelengths in this series of substances; virtually, it is the difference between the location of bands due to sodium D-galacturonan for  $n$  limiting to  $\infty$  and monomeric sodium D-galacturonate. The hypsochromic shift of the electronic transition in central units of uronic acid towards the monomer reflects the change of electronic configuration of the chromophoric group.

The energy of the electronic transition  $n \rightarrow \pi^*$  of carboxyl group of monomer is lower than that of transition of these groups bound inside the chain of the molecule. A like change has been observed also with the electrostatic free enthalpy of dissociation in the series of oligo- and polygalacturonic acids<sup>20</sup> and with binding of various cations to those substances. The counter-ion activity in the molecule of polyanion is substantially lower than that of the corresponding monomer (see *e.g.* the activity of  $\text{Ca}^{2+}$  ions in solutions of calcium oligouronates and glycuronans<sup>13</sup>).

The different behaviour of Ca salts toward Na salts, manifested in the deviation from linearity of relationship under investigation (Fig. 4, curve 2) can be ascribed to a more intense electrostatic effect of a bivalent cation. A distinct spectrum of calcium pectate in solution containing microgel particles<sup>1</sup> reflects the orientation of macromolecules in a conformation close to the solid state with a twofold screw symmetry.

## DISCUSSION

The linear course of the dependence  $[\Theta]_n/n$  on  $(n-2)/n$  shows that the extension of chain is not associated with the change of interactions between the neighbouring units and hence, no conformation change takes place. The environment of each unit

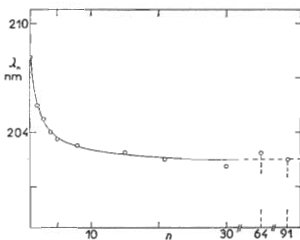


FIG. 3

Relationship between the Wavelength of Maximum of the CD band  $\lambda_n$  and Polymerization Degree  $n$  of Sodium Oligogalacturonates and D-Galacturonans

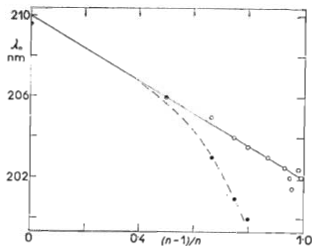


FIG. 4

Plot of the Relationship  $\lambda_n = f((n-1)/n)$  for Sodium Oligogalacturonates and D-Galacturonans  $\circ$  and Calcium Oligogalacturonates  $\bullet$

inside the chain is identically arranged in the space. We exclude therefore the nonbonding intramolecular interactions comprising the more remote units of D-galacturonic acid in the linear macromolecule, as well as the intermolecular nonbonding interactions.

The nonbonding intramolecular interactions involving the mutually more remote D-galacturonic acid units are from steric reasons less probable, since the molecule of homopolymeric D-galacturonan is stretched and considerably rigid. This is evidenced by theoretical calculations of allowed conformations. Due to diaxial glycosidic bonds  $\alpha(1 \rightarrow 4)$  the region of allowed conformations of the linear chain is very narrow and the mutual rotation of the component units is markedly restricted<sup>8,9</sup>. The pectin molecule, in contrast to homopolymeric D-galacturonan, has the sequence of D-galacturonic acid units randomly discontinued in a low extent by L-rhamnose units<sup>16</sup> with glycosidic bonds  $(1 \rightarrow 2, 1 \rightarrow 4)$ . This structural feature could lead to bending of the macromolecule (the so called kinking effect<sup>8,10</sup>). Viscometric measurements, however, show the pectin molecule also to be considerably rigid and stretched<sup>7</sup>, as it further follows from values of the exponent  $a$  in the equation by Mark and Houwink ( $[\eta] = KM^a$ ); ( $a = 1.34$  ( $M_w$ ), ref.<sup>5</sup>;  $1.22$  ( $M_n$ ), ref.<sup>6</sup>;  $1.68$  ( $M_n$ ), ref.<sup>21</sup>). In contrast to these findings, substantially lower values were found when investigating the light scattering of pectin solutions; ( $a = 0.79$ , ref.<sup>22</sup>;  $0.64$ , ref.<sup>17</sup>). These values lead to the conclusion that the pectin molecule has in aqueous solution the conformation of an ordinary random coil. (The cause of different results, obtained by various experimental techniques, was not clarified as yet.)

Intermolecular nonbonding interactions in solution of sodium salts of substances under study (without addition of a further electrolyte) can also be excluded, since the high electric charge of macromolecules does not allow their aggregation. The same holds for solutions of calcium oligogalacturonates  $n = 1-5$ , which are molecularly disperse, as indicated by activity coefficients  $\gamma_{Ca^{2+}}$  estimated in solutions of these substances<sup>13</sup>.

The experimental data evidence the environment of the monomeric structural units inside the chain to be spatially identically arranged. Such a conformation can be realized either in a helical or a random coil arrangement. Due to a considerable rigidity of the pectin chain and especially of homopolymeric D-galacturonan and its high linear charge density we assume that the conformation of sodium D-galacturonan macromolecule is close to a helical arrangement with a screw symmetry of low abundance.

The accordance in chiral properties of sodium and calcium oligomers indicates the conformation of molecules in solution of both these salts to be equal in contrast to results of X-ray diffraction analysis of sodium and calcium pectates. Here, the threefold screw symmetry, similar to that of pectic acid and sodium pectate in solid phase<sup>2</sup>, is likely involved. The twofold screw symmetry of calcium pectate in solid

phase<sup>3</sup> is subject to the formation of aggregates of oriented macromolecules as a consequence of chelate binding of calcium ions (egg-box model<sup>18</sup>). Such an aggregation has also been evidenced in solutions of calcium D-galacturonan in the region of higher polymerization degrees ( $n > 10$ ) ref.<sup>13</sup>.

In our preceding papers<sup>1,19</sup> we showed that at a pure electrostatic binding of cations to glycuronans and derivatives thereof, the CD spectrum is not influenced by the kind of counterions (K, Mg, Ca). The concordance of the chiral properties of sodium and calcium oligogalacturonates (Fig. 2) confirms therefore our previous findings that calcium oligogalacturonates have calcium ions electrostatically bound.

#### REFERENCES

1. Kohn R., Sticzay T.: *This Journal* **42**, 2372 (1977).
2. Palmer K. J., Hartzog M. B.: *J. Amer. Chem. Soc.* **67**, 2122 (1945).
3. Sterling C.: *Biochim. Biophys. Acta* **26**, 186 (1957).
4. Palmer K. J., Ballantyne M.: *J. Amer. Chem. Soc.* **72**, 736 (1950).
5. Owens H. S., Lotzkar H., Schultz T. H., Maclay W. D.: *J. Amer. Chem. Soc.* **68**, 1628 (1946).
6. Orlov S. I.: *Uch. Zap. Saratov. Gos. Univ.* **34**, 46 (1954); *Chem. Abstr.* **53**, 15709 (1959).
7. Smidsrød O., Haug A.: *Biopolymers* **10**, 1213 (1971).
8. Rees D. A., Wight A. W.: *J. Chem. Soc. (B)*, **1971**, 1366.
9. Sathyanarayana B. K., Rao V. S. R.: *Curr. Sci.* **42**, 773 (1973); *Chem. Abstr.* **80**, 48276 (1974).
10. Whittington S. G., Glover R. M., Hallman G. M.: *J. Polymer Sci., Symp. No 42*, 1493 (1973).
11. Morris E. R., Rees D. A., Sanderson G. R., Thom D.: *J. Chem. Soc., Perkin Trans. 2*, **1975**, 1418.
12. Kohn R.: *Carbohyd. Res.* **20**, 351 (1971).
13. Kohn R., Luknár O.: *This Journal* **42**, 731 (1977).
14. Mukherjee S., Marchessault R. H., Sarko A.: *Biopolymers* **11**, 291 (1972).
15. Freudenberg K., Kuhn W., Dürr W., Bolz F., Steinbrunn G.: *Ber. Deut. Chem. Ges.* **63**, 1510 (1930).
16. Cook C. M. W., Stoddart R. W.: *Surface Carbohydrates of the Eukaryotic Cell*, p. 182. Academic Press, London—New York 1973.
17. Kawabata A., Sawayama S.: *J. Agr. Chem. Soc. Jap.* **51**, 15 (1977).
18. Grant G. T., Morris E. R., Rees D. A., Smith P. J. C., Thom D.: *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **32**, 195 (1973).
19. Kohn R., Malovíková A.: *This Journal* **43**, 1709 (1978).
20. Kohn R., Malovíková A.: Unpublished results.
21. Fritzsche P., Lehmann I., Dongowski G., Bock W.: *Faserforsch. Textiltech.* **28**, 543 (1977).
22. Berth G., Anger H., Linow F.: *Nahrung* **21**, 939 (1977).

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