

BINDING OF CALCIUM IONS TO 2,3-DICARBOXY DERIVATIVES OF PECTIC ACID

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Using a two-step oxidation of pectic acid (85% of D-galacturonan in the preparation) a series of 2,3-dicarboxy derivatives (Na^+ form) was prepared containing 4.2 to 8.5 mmol of carboxyl groups per gram (COOH g^{-1}). After the centrifugation of the diluted suspensions of the Ca-salts of these polyacids the activity of the calcium counterions ($a_{\text{Ca}^{2+}}$) was determined using the metallochromic indicator (tetramethylmurexide) and the activity coefficient $\gamma_{\text{Ca}^{2+}}$ was calculated. Using potentiometric titrations of polyacids with potassium and calcium hydroxides the decrease in electrostatic free enthalpy, $\Delta(G_{\text{el}}/N)_{\text{K}}^{\text{Ca}}$, of the cation exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ was determined. In the course of the oxidation, degradation of the macromolecules took place, characterized by the limit viscosity number $[\eta]$. In spite of the considerable degradation, preparations with higher oxidation degrees display a high selectivity in the exchange of cations $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$, similar to that determined recently by us in 2,3-dicarboxy derivatives of starch and amylose. The strong binding of Ca^{2+} ions to the investigated substances is also documented by very low activity coefficient values, $\gamma_{\text{Ca}^{2+}}$, ranging from 0.057 to 0.037.

Pectin is characterized by the ability to bind and exchange cations; it behaves as a highly selective cation exchanger. In recent years we investigated the binding and the exchange of a number of divalent, essential, and toxic cations in pectin and its oligomeric fragments (*cf.* refs¹⁻⁶). The data obtained threw some light on the optimum conditions for the application of pectin as a prophylactic substance and a remedy for poisoning by metal cations (lead, cadmium, copper).

The capacity of the binding of cations to pectin is given by the content of D-galacturonan and the degree of esterification (E) of the carboxyl groups with methanol. Sodium pectate (completely deesterified pectin, $E 0\%$) contains about 4.0–4.5 mmol of carboxyl groups in 1 g of the preparation. The capacity of the binding of cations to the pectate can be increased substantially by specific oxidation of the D-galacturonic acid units in the pectin molecule, under formation of a pair of new carboxyl groups with $\text{C}_{(2)}$ and $\text{C}_{(3)}$ atoms. One of the well elaborated methods of oxidation of acid polysaccharides is periodate oxidation during which secondary alcoholic groups of saccharides are oxidized to aldehyde groups under formation of dialdehyde derivatives of the starting substances (see refs⁷⁻¹⁰). In a further oxidation step various oxidation reagents¹¹ were used, as for example sodium chlorite, bromine, some nitrogen oxides, *etc.*, leading to corresponding dicarboxy derivatives in a practically quantitative yield.

Carboxy derivatives of polysaccharides are used in industry and also in medicine. Carboxy derivatives of starch have proved a good sequestration additive to detergents. Carboxy derivatives of pectin have been prepared for the same purpose by its oxidation with chlorine in alkaline medium¹². Carboxy derivatives of starch and amylose are used owing to their bactericidal and antiviral effect and the ability to bind ammonia (for a survey see ref.¹³).

From the point of view of the binding of cations the 2,3-dicarboxy derivatives of pectic acid appear interesting in consequence of the high local charge density, due to the existence of three carboxyl groups on one oxidized D-galacturonic acid unit in the macromolecule chain. So far little attention has been devoted to the binding of cations on 2,3-dicarboxy derivatives of pectic acid. Only the studies by Ashubaeva and coworkers¹¹ are known, which deal mainly with the preparation of these substances. They studied the binding of several divalent cations to 2,3-dicarboxy derivatives of pectic acid by means of conductometric titrations and IR spectroscopy. The results led to the conclusion that the degree of oxidation of pectin preparations has no substantial effect on the stoichiometry of the binding of cations, especially in the case of binding of Ca^{2+} and Mg^{2+} ions. The authors explain¹¹ the slightly higher proportion of the bound cations, found in other cases, by the interaction of the cations with the hydroxyl groups of polyuronates.

In the present paper we endeavour to elucidate the effect of the oxidation degree of 2,3-dicarboxy derivatives of pectic acid on the binding of calcium ions and the selectivity of the exchange of the cations $\text{Ca}^{2+} \rightarrow 2 \text{K}^{+}$ in these derivatives.

EXPERIMENTAL

Material

The starting potassium pectate which was used for the preparation of 2,3-dicarboxy derivatives of pectic acid was prepared from citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns Pektinfabrik, Denmark) using alkaline deesterification in a suspension in 60% ethanol. The preparation was washed with 60% and 96% neutral ethanol and dried at 60°C. It contained 84.9% of potassium D-galacturonan and 15.1% of neutral saccharides, bound predominantly in the form of short side chains. Limit viscosity number $[\eta] = 133 \text{ cm}^3 \text{ g}^{-1}$.

2,3-Dicarboxy Derivatives of Pectic Acid

Various oxidized preparations were obtained by suitably increasing doses of oxidants and time of oxidation.

Potassium pectate (10 g) was suspended in water (100–150 cm³) and a solution of NaIO_4 (2 to 20 g in 400 cm³ of water, depending on the required degree of oxidation) was added under continuous stirring. Oxidation took place at room temperature over 1 to 48 h. The oxidation product was precipitated by a double volume of acetone and allowed to stand for 24 h; the precipitate was then filtered off on a fritted filter and washed with acetone. The crude product obtained in this manner was a dialdehyde, containing salts, suitable for further use without purification.

The suspension of dialdehyde in water was mixed with a solution of NaClO_2 (8 to 40 g in 200 cm^3 of water) and the pH of the solution was adjusted by slow addition of concentrated acetic acid to pH 3.6–3.9, under constant stirring and cooling ($\sim 10^\circ\text{C}$). The mixture was stirred 1/2 to 3 h or further allowed to stand without stirring, so that the total reaction time was 2 to 24 h. Chlorine was eliminated by bubbling nitrogen through the mixture, the pH of the solution was adjusted by addition of sodium hydroxide to 4.5–4.8 and the product was precipitated with a double amount of methanol. The preparation was filtered off, washed with 96% ethanol, dissolved in a small amount of water and the pH value of the solution was adjusted by addition of sodium hydroxide to 8.5–8.8. The solution thus adjusted was dialysed against water (for 48 h). The dialysis course was followed conductometrically and also by testing the presence of IO_3^- ions. After dialysis the solution was evaporated on a vacuum rotatory evaporator at 40°C . The yields of individual preparations ranged from 6 to 8 g.

Solutions of carbonate free 0.05M KOH and 0.05M NaOH were used, further a saturated 0.021M $\text{Ca}(\text{OH})_2$ solution, a 0.01M Complexon IV solution, tetramethylmurexide synthesized by us^{14,15}, and redistilled water free of atmospheric CO_2 . All the chemicals used were of analytical grade.

Analytical Methods

The content of free carboxyl groups was determined by alkalimetry. The dilute solution of carboxy derivative in Na^+ form was percolated through a Dowex 50 W \times 2 column (H^+ form) and the eluate was titrated potentiometrically with 0.05M KOH. The total content of the carboxyl groups in the samples of 2,3-dicarboxy derivatives was determined analogously after the opening of the small fraction of the lactones present in the preparation by addition of a mild excess of hydroxide ($5\text{--}10 \text{ mmol (KOH) dm}^{-3}$) in closed vessels at room temperature for 18 h. The excess of hydroxide was eliminated by percolation through a column of the above-mentioned cation exchanger. The resulting solution of polyacid was then titrated potentiometrically with a 0.05M KOH solution to equivalence point.

The limit viscosity number $[\eta]$ of the sodium salts of 2,3-dicarboxy derivatives of pectic acid was determined by means of an Ubbelohde viscosimeter in a medium of 0.15M NaCl–0.005M sodium oxalate at $25.0 \pm 0.1^\circ\text{C}$.

The decrease in the electrostatic Gibb's free enthalpy $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$, connected with the exchange of cations $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ in 2,3-dicarboxy derivatives of pectic acid was determined by potentiometric titration of polyacids of $3.00 \text{ mmol (COOH) dm}^{-3}$ concentration with the hydroxides 0.05M KOH and 0.021M $\text{Ca}(\text{OH})_2$. For both titrations the $\text{pH} = f(\text{DN})$ plot was constructed, where DN is the degree of neutralization. The change of the electrostatic Gibb's free enthalpy, referred to 1 mol of carboxyl groups, is given by the area limited by both titration curves¹⁶; the area was determined planimetrically.

The activity of the calcium ions ($a_{\text{Ca}^{2+}}$) and the activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of the calcium salts of 2,3-dicarboxy derivatives of pectic acid were determined using the method of metallochromic indicator (tetramethylmurexide)^{14,15}. The investigated solutions of the sodium salts of 2,3-dicarboxy derivatives of pectic acid, converted to corresponding polyacids (Dowex 50W \times 2; H^+), were neutralized with a calcium hydroxide solution to equivalence point. Since during neutralization a partial precipitation of the calcium salt took place in all samples, the suspension was centrifuged at $15\,000 \text{ g}$ for 20 min. In the supernatant which was used for the determination of the activities of calcium ions the total amount of calcium was determined by chelometric titration with Complexon IV, under photometric indication of the equivalence point (Zeiss interference filter, GDR, IF 600 nm, murexide as indicator). The activity $a_{\text{Ca}^{2+}}$ was determined without addition of a secondary electrolyte.

The IR spectra of the potassium salt of 2,3-dicarboxy derivatives of pectic acid were determined by the KBr technique on Perkin-Elmer 577 and UR-20 spectrometers; for details see ref.¹⁷. The ¹³C NMR spectra of the starting potassium pectate (a solution of 0.15 mol (COOK) dm⁻³ concentration in ²H₂O) and 2,3-dicarboxy derivatives of pectic acid (0.50 mol (COONa) dm⁻³; ²H₂O) were measured on a Nicolet 360 MHz, U.S.A., instrument, using dioxane as external standard. The potentiometric titrations were carried out with a Radelkis OP-208 potentiometer, using combined glass electrode OP-0808P. The spectrophotometric measurements were carried out on a UVISPEC-Hilger spectrometer.

RESULTS AND DISCUSSION

Characterization of 2,3-Dicarboxy Derivatives of Pectic Acid

Using two-step oxidation of potassium pectate (completely deesterified pectin, *E* 0%) 2,3-dicarboxy derivatives of pectic acid with different oxidation degrees and different binding capacity for cations were prepared; the final products were obtained in the form of Na salts. In Fig. 1 a segment of the macromolecule of non-oxidized pectic acid (D-galacturonan) (a) is represented together with its oxidation product, 2,3-dicarboxy derivative (b).

On periodate oxidation of the pectate not all the D-galacturonic acid units in the macromolecule chain are oxidized to the corresponding dialdehyde. The reaction

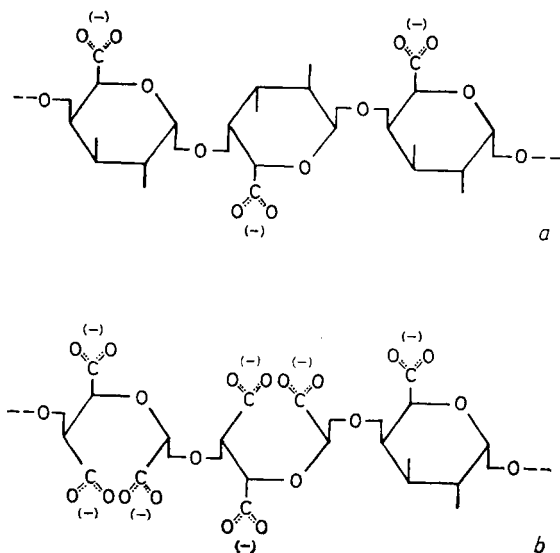


FIG. 1

Segment of the pectic acid macromolecule (a) and of partially oxidized pectic acid — 2,3-dicarboxy derivative (b)

stops at the consumption of 0.64 ± 0.01 mol NaIO_4 per one internal uronic acid unit¹⁰. The author of the paper mentioned investigated the reaction conditions of the periodate oxidation of sodium pectate and pectinates in detail ($E > 0\%$). He found that at the beginning the oxidation proceeds under formation of dialdehyde of the D-galacturonic acid units randomly distributed in the macromolecule chain. However, the oxidative reaction is then gradually inhibited by a unidirectional formation of a hemiacetal between the oxidized and the neighbouring non-oxidized unit of uronic acid, in contrast to the periodate oxidation of alginates, where a bilateral formation of hemiacetal⁹ takes place. The study of the conformational aspects of the hemiacetal formation¹⁰ led to the conclusion that the hemiacetal in oxidized pectin is formed between atom $C_{(2)}$ of the oxidized D-galacturonic acid unit and the atom $C_{(3)}$ of the non-oxidized unit, in the direction from the non-reducing to the reducing end of the molecule. To this limit dialdehyde corresponds – after oxidation of the aldehyde groups to carboxyl groups – the 2,3-dicarboxy derivative of D-galacturonan with a maximum content of carboxy groups of 9.29 mmol (COONa) in 1 g of the dry weight of the preparation.

2,3-Dicarboxy derivatives of pectic acid (samples 2 to 12) are characterized in Table I. The starting preparation of potassium pectate (Sample 1) contained 85% of potassium D-galacturonan and 15% of neutral saccharides bound predominantly in short side chains. The neutral saccharides also undergo oxidation with periodate and chlorite and therefore the oxidation degree of individual D-galacturonic acid units cannot be calculated accurately from the total content of the carboxyl groups. For this reason we express the degree of oxidation of pectic acid only by the content of carboxyl groups in individual oxidized preparations (Table I, mmol (COOH) g^{-1}).

The content of free carboxyl groups in original preparations (series *a*) is slightly lower than the total content of carboxyl groups in the preparations after the opening of the lactones by means of hydroxide (series *b*). This difference corresponds to the presence of lactones in the original samples in an amount from 3 to 10% of the total content of carboxyl groups; on average approximately 6%.

During the oxidation of potassium pectate a considerable degradation of the macromolecule chain takes place. This phenomenon has already been observed and investigated in detail under the conditions of periodate oxidation¹¹. Simultaneously with the oxidation of glycol grouping on $C_{(2)}$ and $C_{(3)}$ of D-galacturonic acid units the splitting of the glycosidic bonds and the formation of the terminal aldehyde groups also take place. The degree of degradation is proportional to the degree of oxidation of the preparations, *i.e.* the amount of the oxidant used, as well as the reaction time. The limit viscosity number $[\eta]$ as a measure of relative molecular weight of 2,3-dicarboxy derivatives of pectic acid (Table I) dropped from the starting value $[\eta] = 133 \text{ cm}^3 \text{ g}^{-1}$ in non-oxidized pectate to the value $[\eta] = 5 \text{ cm}^3 \text{ g}^{-1}$ in the sample with the highest content of carboxyl groups. From the course of the corresponding function $[\eta] = f(\text{COOH})$ (Fig. 2, curve 3) it is evident that the strongest degradation

TABLE I

Characterization of 2,3-dicarboxy derivatives of pectic acid (Na⁺ form). Change of the electrostatic free enthalpy during the exchange of cations Ca²⁺ → 2 K⁺

Sample	(COOH) mmol g ⁻¹		[η] cm ³ g ⁻¹	Δ(G _{e1} /N) _K ^{Ca} kJ (COOH) ⁻¹	
	a	b		a	b
1 ^c	4.23 ± 0.01	—	133	-3.09 ± 0.04	—
2	4.80 ± 0.06	5.14 ± 0.09	31	-5.43 ± 0.07	-5.72 ± 0.26
3	4.77 ± 0.02	5.20 ± 0.05	32	-5.61 ± 0.28	-6.08 ± 0.26
4	5.22 ± 0.01	5.47 ± 0.05	27	-6.55 ± 0.46	-6.36 ± 0.10
5	5.48 ± 0.03	6.08 ± 0.08	19	-6.81 ± 0.18	-6.80 ± 0.25
6	6.76 ± 0.05	7.30 ± 0.02	14	-7.89 ± 0.30	-7.63 ± 0.11
7	7.25 ± 0.06	7.50 ± 0.05	13	-8.80 ± 0.32	-8.33 ± 0.10
8	7.38 ± 0.04	7.56 ± 0.04	—	-8.69 ± 0.08	-8.68 ± 0.23
9	7.16 ± 0.02	7.59 ± 0.02	9	-8.22 ± 0.13	-8.49 ± 0.15
10	7.35 ± 0.14	7.71 ± 0.26	—	-8.59 ± 0.10	-8.60 ± 0.38
11	7.39 ± 0.09	7.74 ± 0.11	9	-8.39 ± 0.53	-8.22 ± 0.05
12	8.20 ± 0.02	8.46 ± 0.04	5	-8.81 ± 0.05	-8.49 ± 0.26

^a Original preparations containing a small portion of lactones; ^b preparations after hydrolysis of lactones; ^c starting sample of non-oxidized potassium pectate.

takes place just at the initial stage of the oxidation of the pectate to its 2,3-dicarboxy derivative. In spite of such a considerable degradation of the macromolecules all the

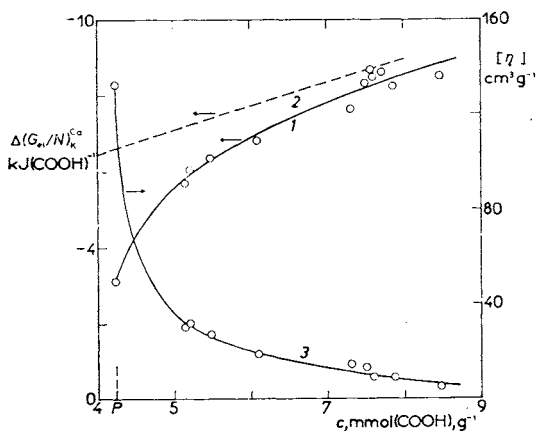


FIG. 2

Dependence of the electrostatic free enthalpy of the cation exchange Ca²⁺ → 2 K⁺, Δ(G_{e1}/N)_K^{Ca}, of the 2,3-dicarboxy derivatives of pectic acid on the degree of its oxidation. *c* content of carboxyl groups in the preparation; *P* starting pectic acid; [η] limit viscosity number of solutions of Na-salts of 2,3-dicarboxy derivatives of pectic acid; 1, 2 Δ(G_{e1}/N)_K^{Ca} = *f*(*c*) for 2,3-dicarboxy derivatives of pectic acid or starch; 3 [η] = *f*(*c*)

preparations investigated displayed a high selectivity in the cation exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ which increased with increasing oxidation degree of pectic acid.

2,3-Dicarboxy derivatives of pectic acid were further characterized by means of their IR and ^{13}C NMR spectra. The IR spectra of the prepared derivatives were described in detail in a separate communication¹⁷. The spectra were interpreted mainly from the point of view of structural changes provoked in the pectic acid molecule by its oxidation, *i.e.* by the opening of the pyran ring of the D-galacturonic acid units and the formation of a couple of new carboxyl groups. Both these changes in the structure of the starting pectic acid were distinctly reflected in the IR spectra of the substances investigated (for details see ref.¹⁷).

The ^{13}C NMR spectra of 2,3-dicarboxy derivatives of pectic acid are not described in literature. Fig. 3 represents the spectrum of the starting potassium pectate. The resonance signals obtained for individual pyran ring carbons of the D-galacturonic acid units in the potassium pectate molecule are given in ppm: $\text{C}_{(1)}$ 99.75, $\text{C}_{(2)}$ 68.97, $\text{C}_{(3)}$ 69.64, $\text{C}_{(4)}$ 78.70, $\text{C}_{(5)}$ 72.10, $\text{C}_{(6)}$ 176.16. They are well comparable with the chemical shift values of the carbon atoms in sodium D-galacturonan¹⁸. Oxidation

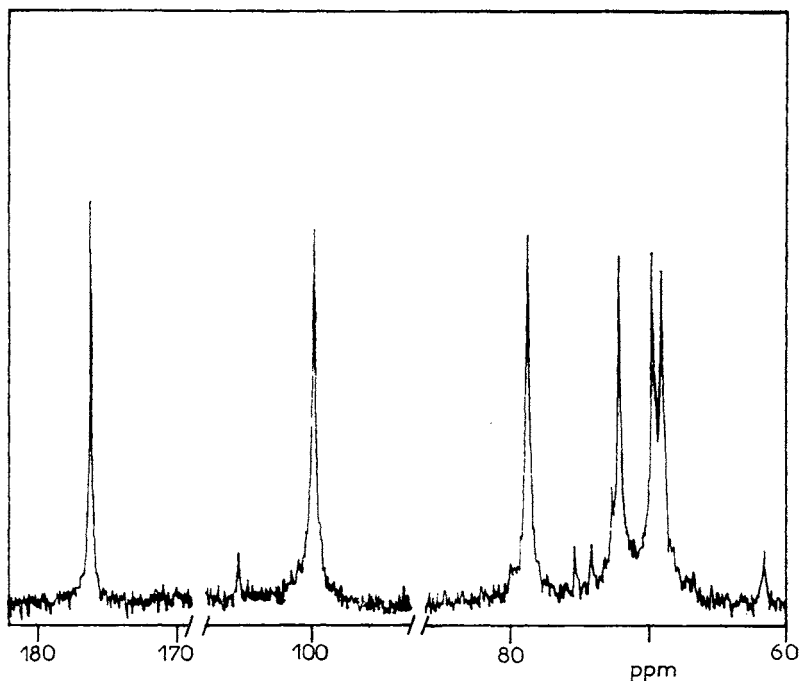


FIG. 3
 ^{13}C NMR spectrum of the starting potassium pectate sample 1 (Table I); $c_{\text{COOH}} = 0.15 \text{ mol dm}^{-3}$

of pectic acid under formation of its 2,3-dicarboxy derivatives brings about a distinct change in the ^{13}C NMR spectra (Fig. 4a, sample 5; 4b, sample 9). As expected, in the spectra of oxidized pectic acid derivatives new signals appear in addition to the original partly shifted resonance signals, predominantly in the region of the $\text{C}_{(1)}$, $\text{C}_{(4)}$, and $\text{C}_{(6)}$ atoms resonance. As is evident from comparison of samples 5 and 9 the spectrum becomes more complex with increasing degree of oxidation.

The Binding of Calcium Ions to 2,3-Dicarboxy Derivatives of Pectic Acid

The binding of Ca^{2+} ions and the selectivity of the $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ exchange in solutions of 2,3-dicarboxy derivatives of pectic acid of various degrees of oxidation was evaluated on the basis of the activities of calcium counterions a_{Ca^+} bound to the carboxyl groups of these derivatives, their activity coefficient $\gamma_{\text{Ca}^{2+}}$ and on the basis of the change of electrostatic free enthalpy, $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$, of the exchange of $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ ions.

The exchange of K^+ ions, which are relatively weakly bound to the carboxyl groups of 2,3-dicarboxy derivatives of pectic acid, for the substantially more firmly bound

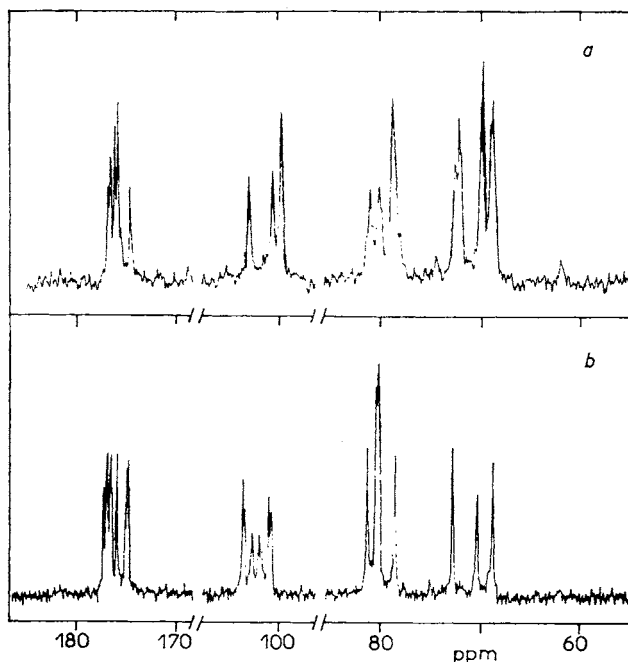


FIG. 4

^{13}C NMR spectrum of 2,3-dicarboxy derivative of pectic acid. a sample 5; b sample 9; $c_{\text{COOH}} = 0.50 \text{ mol dm}^{-3}$

Ca^{2+} ions manifests itself in the decrease of the electrostatic Gibbs free enthalpy; hence, the change of $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$ is a measure of the selectivity of the cation exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$. Its value is proportional to the area (A) limited by the potentiometric titration curves of neutralization of corresponding polyacids with potassium and calcium hydroxide¹⁶. Fig. 5 shows — for the sake of comparison — titration curves of the starting non-oxidized pectic acid (sample 1, diagram *a*) and its 2,3-dicarboxy derivative with the highest carboxyl group content (sample 12, diagram *b*). The ratio of the areas A_b/A_a shows that the decrease of the electrostatic free enthalpy of the cation exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ in the sample with the highest oxidation degree is approximately 2.8 times higher than in the case of the starting pectic acid. As shown earlier¹⁹ the pectic acid itself behaves as a highly selective cation exchanger for a number of divalent cations, $\text{M}^{2+} \rightarrow 2 \text{K}^+$ ($\text{M} = \text{Mg}, \text{Ca}, \text{Sr}, \text{Co}, \text{Cu}, \text{Pb}$). Taking these facts into consideration in the interpretation of the $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$ values determined in 2,3-dicarboxy derivatives of pectic acid (Table I, samples 2–12) we may state unambiguously that these derivatives are highly selective with respect to Ca^{2+} ions.

The selectivity of the ion exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$ increases with the carboxyl groups content in preparations, *i.e.* with the degree of oxidation of pectic acid (Fig. 2, curve 1) and is not affected under the given experimental conditions by the relatively considerable degradation of the pectic acid during its oxidation either. The degree of degradation of pectic acid molecule is indirectly proportional to the limit viscosity number $[\eta]$, determined in the solutions of sodium salts of 2,3-dicarboxy derivatives of pectic acid (Fig. 2, curve 3). Very similar results were obtained in both sample series, *i.e.* in original preparations containing a small proportion of lactones (series *a*) and the preparations in which the lactones were eliminated under the effect of an alkaline medium (series *b*) (Table I).

The high selectivity of the cation exchange remains preserved in strongly degraded

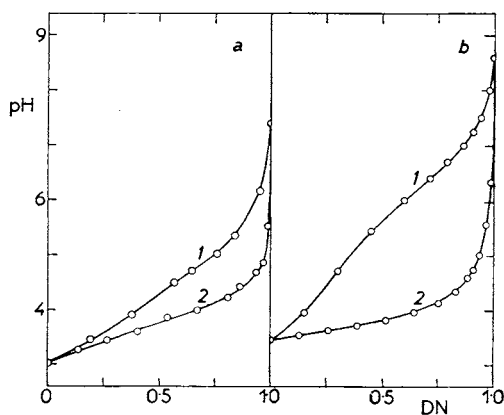


FIG. 5

Determination of the electrostatic free enthalpy of the cation exchange $\text{Ca}^{2+} \rightarrow 2 \text{K}^+$, $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$, in solutions of pectic acid and its 2,3-dicarboxy derivative, $c_{\text{COOH}} = 3.00 \text{ mmol dm}^{-3}$. *a* starting pectic acid (sample 1); *b* its 2,3-dicarboxy derivative (sample 12); 1 titration with KOH ; 2 titration with Ca(OH)_2 ; DN degree of neutralization; A area proportional to the value of $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$

samples, $[\eta] = 5 - 10 \text{ cm}^3 \text{ g}^{-1}$, where we are probably already in the region of oligomeric fragments. We have demonstrated in preceding studies^{20,21} that the strength of binding of Ca^{2+} ions to polymeric uronans (D-galacturonan, L-guluronan, D-mannuronan) does not depend on the chain length of the macromolecule if the polymerization degree of the uronan is higher than 30. The high cation exchange selectivity of strongly degraded 2,3-dicarboxy derivatives of pectic acid (samples 9–12) may be ascribed to the high local density of the charge on the oxidized D-galacturonic acid unit, containing 3 carboxyl groups (Fig. 1b).

The pair of $\text{C}_{(2)}\text{OOH}$ and $\text{C}_{(3)}\text{OOH}$ groups principally contributed to the strong binding of Ca^{2+} ions. In consequence of the opening of the pyran ring during periodate oxidation of the D-galacturonic acid unit the chain of the pectic acid macromolecule becomes flexible and both carboxyl groups may get into a spatial arrangement which is optimal for a strong binding of the Ca^{2+} ions. The data in Fig. 2 are completed by $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}}$ values determined for 2,3-dicarboxy derivatives of starch (curve 2) which contain only these pairs of carboxyl groups. From a comparison of the course of curves 1 and 2 it is evident that the carboxyl groups $\text{C}_{(2)}\text{OOH}$ and $\text{C}_{(3)}\text{OOH}$ bind at an equal carboxyl group content in the preparation the Ca^{2+} ions much more strongly than the carboxyl group of uronic acid ($\text{C}_{(6)}\text{OOH}$). Pectic acid (point P) displays a substantially lower selectivity of the cation exchange ($\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}} = -3.09 \text{ kJ (COOH)}^{-1}$) in comparison with the 2,3-dicarboxy derivatives of starch with the same content of carboxyl groups in the preparation, point P; $\Delta(G_{e1}/N)_{\text{K}}^{\text{Ca}} = -6.6 \text{ kJ (COOH)}^{-1}$. With progressing oxidation of pectic acid in which oxidized D-galacturonic acid units multiply (comprising 3-carboxyl groups), this difference diminishes. Unfortunately, the difference in the binding of calcium ions to 2,3,6-tricarboxy and 2,3-dicarboxy derivatives of polysaccharides cannot be deduced from the experimental data, because the 2,3-dicarboxy derivative of pectic acid with the highest content of carboxyl groups (sample 12) represents a compound in which only 60% of the D-galacturonic acid units is oxidized. (The maximal degree of pectic acid oxidation by two-step oxidation with periodate and chlorite corresponds to an oxidation of 64% of the uronic acid units¹⁰.)

We further investigated the binding of Ca^{2+} ions to 2,3-dicarboxy derivatives of pectic acid on the basis of the activity coefficient of calcium counterions ($\gamma_{\text{Ca}^{2+}}$), bound to the carboxyl groups of these derivatives. The measurements were carried out with the original samples (series a). The total concentration of the Ca^{2+} ions added after the achievement of the equivalence point in the neutralization of corresponding polyacids with calcium hydroxide was in the $1.85 - 2.49 \text{ mmol (Ca}^{2+}) \text{ dm}^{-3}$ range (for the $c_{0,\text{Ca}^{2+}}$ values). In all the samples of 2,3-dicarboxy derivatives of pectic acid investigated a partial coagulation of the calcium salt took place during the neutralization. After the elimination of the suspended particles the total concentration of calcium (c_{Ca}) was determined in the supernatant, as well as the activity of the calcium ions ($a_{\text{Ca}^{2+}}$) and the activity coefficient $\gamma_{\text{Ca}^{2+}}$ was calculated (Table II).

The activity coefficient of the Ca^{2+} ions of the starting non-oxidized pectic acid (sample 1, $\gamma_{\text{Ca}^{2+}} = 0.073$) is anomalously low in consequence of the considerably strong intermolecular bond of calcium of the chelate type¹. (The value $\gamma_{\text{Ca}^{2+}} = 0.30$, ref.²², corresponds to the intramolecular electrostatic binding of Ca^{2+} ions to the D-galacturonan chain.) The activity coefficients $\gamma_{\text{Ca}^{2+}}$ determined in the solutions of calcium salts of 2,3-dicarboxy derivatives of pectic acid ($\gamma_{\text{Ca}^{2+}} = 0.057-0.037$) are still lower, they decrease with the increasing oxidation degree of pectic acid (Fig. 6), and represent proof of the very strong binding of Ca^{2+} ions in these systems, due to

TABLE II

Binding of calcium ions to 2,3-dicarboxy derivatives of potassium pectate

Sample	(COOH) ^a mmol g ⁻¹	$c_{\text{Ca}^{2+}}$ ^b mmol dm ⁻³	$\gamma_{\text{Ca}^{2+}}$
1 ^c	4.23 ± 0.01	1.009 ± 0.094	0.073 ± 0.003
2	4.80 ± 0.06	1.148 ± 0.050	0.048 ± 0.004
3	4.77 ± 0.02	1.110 ± 0.059	0.057 ± 0.004
4	5.22 ± 0.01	1.274 ± 0.130	0.051 ± 0.002
5	5.48 ± 0.03	1.267 ± 0.090	0.057 ± 0.002
6	6.76 ± 0.05	1.248 ± 0.061	0.038 ± 0.003
9	7.16 ± 0.02	0.902 ± 0.039	0.047 ± 0.002
8	7.38 ± 0.04	1.060 ± 0.027	0.037 ± 0.006
12	8.20 ± 0.02	1.078 ± 0.032	0.041 ± 0.000

^a Content of carboxyl groups in preparations (Na^+ form); ^b $c_{\text{O},\text{Ca}^{2+}} = 1.85-2.49$ mmol (Ca^{2+}) · dm⁻³; ^c starting sample of non-oxidized potassium pectate.

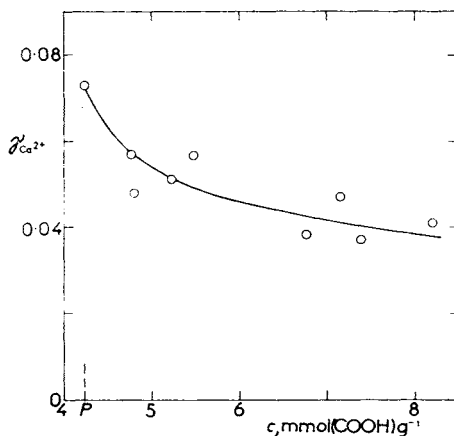


FIG. 6

Dependence of the activity coefficient $\gamma_{\text{Ca}^{2+}}$ in solutions of the calcium salt of 2,3-dicarboxy derivatives of pectic acid on the degree of its oxidation. c content of carboxyl groups in the preparation; P starting pectic acid

the high local density of the charge of the oxidized uronic acid units. The reason is probably both the intramolecular binding of the Ca^{2+} ions to the pair of the carboxyl groups $\text{C}_{(2)}\text{OOH}$ and $\text{C}_{(3)}\text{OOH}$, and the intermolecular binding to the carboxyl groups $\text{C}_{(6)}\text{OOH}$ of the uronic acid units, because the preparations still contain a relatively large amount of non-oxidized D-galacturonic acid units. The low values of the activity coefficient $\gamma_{\text{Ca}^{2+}}$ indicate the formation of a Ca-complex with a very low dissociation degree. The determination of the mode of binding and the type of complex of Ca^{2+} ions with the carboxyl groups of 2,3 dicarboxy derivatives of pectic acid requires further systematic studies.

On the basis of the demonstrated high selectivity of 2,3-dicarboxy derivatives of pectic acid for the Ca^{2+} ions it may be justly assumed that these derivatives will also be highly selective towards other divalent cations, similarly as in the case of 2,3-dicarboxy derivatives of starch and amylose²³. A practical exploitation of 2,3-dicarboxy derivatives of pectic acid may be expected primarily in preparations with a low degree of oxidation which are still biologically degradable.

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