Infrared spectra of 2,3-dicarboxy derivatives of pectic acid

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Received 1 November 1984

A series of 2,3-dicarboxy derivatives of sodium pectate (oxidation degree *DO* 0.0 to 0.5) was obtained by a two-step oxidation of potassium pectate by $NaIO_4$ and $NaClO_2$; their IR spectra were measured using a KBr technique. The spectra were interpreted considering mainly the structural changes evoked by oxidation of pectic acid, *i.e.* by opening of the pyran ring of D-galacturonic acid units and formation of couples of new (C-2) and (C-3) carboxyl groups. Properties of these carboxyl groups are different from those of uronic acids.

Посредством двухстадийного окисления пектата калия под действием $NaIO_4$ и $NaClO_2$ был получен ряд производных пектиновой кислоты в форме натриевых солей со степенью окисления *DO* от 0,0 до 0,5. ИК-спектры этих веществ были записаны с применением KBr-техники. Спектры были интерпретированы, в основном, с точки зрения структурных изменений, вызванных в молекуле пектиновой кислоты в результате ее окисления, то есть открытия пиранового цикла единиц D-галактуроновой кислоты и образования пар новых карбоксильных групп C-2 и C-3. Их свойства отличаются от свойств карбоксильной группы уроновой кислоты.

One of the considerable physiological properties of pectin is its ability to bind and exchange essential and toxic cations. This carboxylic polysaccharide behaves as a highly selective cation exchanger. The ion-exchange capacity of pectin depends on the content of D-galacturonic acid units in the pectin macromolecule and on the esterification degree (*DE*) of carboxyl groups by methanol. The totally deesterified pectin (*DE* 0 %) in the form of sodium salt contains roughly 4.0—4.5 milimoles of (COONa) g^{-1}. The ion-exchange capacity of pectic acid can even be substantially enhanced by specific oxidation during which opening of the pyran ring of D-galacturonic acid units is taking place. Couples of new (C-2) and (C-3) carboxyl groups markedly influence both the binding and the exchange of cations [1—3].

The IR spectrometry has proved to be an effective tool for characterization of pectic substances (determination of esterification and acetylation degrees, binding of water as well as cations to pectin). Papers referring to this problem are listed in monograph [4].

The IR spectrometry has also been successful to characterize oxidized polysaccharides (mono-, di-, tricarboxy celluloses) and the binding of cations to these derivatives, *cf. e.g.* [5, 6]. 2,3-Dicarboxy derivatives of pectic acid were investigated by IR spectrometry especially for interaction of various bivalent cations with carboxyl groups [1, 2]. So far, no detailed study concerning the IR spectra in the whole spectral range reflecting the structural changes due to oxidation of pectic acid has appeared. The aim of our endeavour was to describe and interprete the spectra of 2,3-dicarboxy derivatives of pectic acid in relation to their oxidation degree, as a considerable characteristics of these pectin derivatives having interesting applications both in industry and medicine.

Experimental

Preparation of derivatives and analytical methods

The starting potassium pectate (esterification degree *DE* 0 %) was prepared from the commercial pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns Pektinfabrik) by alkaline deesterification in suspension in a 60 % ethanol. The preparation was stepwise washed with 60 % and 96 % ethanol and dried under reduced pressure at temperature not exceeding 60 °C. This preparation contained in dry matter 85 % of potassium D-galacturonan and 15 % of neutral saccharides linked predominantly in form of short side chains. The limiting viscosity number $[\eta] = 133$ cm^3 g^{-1}.

2,3-Dicarboxy derivatives

Potassium pectate (10 g) was suspended in water (100—150 cm^3). Solution of $NaIO_4$ (2—20 g) in water (400 cm^3) was added with stirring to this suspension; oxidation lasted 1 to 48 h at room temperature. The reaction was stopped by addition of ethylene glycol (5 cm^3). The oxidation product was after 30 min precipitated by addition of acetone in a 1:2 ratio; the precipitate was filtered off through a sintered glass filter after standing for 24 h, and washed with acetone. The crude dialdehyde of pectate containing still a small amount of salts obtained by this process was employed for further oxidation.

A solution of $NaClO_2$ (8 to 40 g) in water (200 cm^3) was added to the suspension of dialdehyde; pH value of the solution was adjusted by successive addition of concentrated acetic acid to 3.6—3.9 under permanent cooling to 10 °C. The mixture was stirred for additional few hours and then left to stand (the reaction time was 2 to 24 h). The pH value was adjusted by sodium hydroxide to 4.5—4.8, chlorine was removed from the mixture by

a stream of nitrogen and the product was precipitated by addition of methanol in a 1 : 2 ratio. The preparation was filtered off, washed with 96 % ethanol, dissolved in a small amount of water, the pH was adjusted with sodium hydroxide to 8.5—8.8 and the solution dialyzed against redistilled water (48 h). The dialysis was monitored conductometrically and by testing the presence of IO_3^- ions. After dialysis the solution was evaporated to dryness under reduced pressure at 40 °C. Yields of the individual preparations varied within 6—8 g (samples of series *a*).

A small portion of lactones in preparations of 2,3-dicarboxy derivatives of pectic acid was removed by alkali metal hydroxide. Solutions of 2,3-dicarboxy derivatives of pectic acid (0.2 %, sodium salt) in 0.03 mol dm^{-3} NaOH were left to stand at an ambient temperature for 16 h in hermetically closed vessels. The alkaline solution was then percolated through a cation exchanger Dowex 50W X 2 (H^+) packed column and the resulting solution of polyacid was neutralized with 0.05 mol dm^{-3} NaOH to the point of equivalence; preparations thereof were obtained by freeze-drying (samples of series *b*).

Analytical methods

The carboxyl groups of derivatives obtained were determined alkalimetrically. A diluted solution of 2,3-dicarboxy derivatives of sodium pectate was percolated through a Dowex 50W X 2 (H^+) packed column; the eluate was titrated potentiometrically with 0.05 mol dm^{-3} KOH. The mentioned NaOH and KOH solutions were carbonate-free, the redistilled water did not contain atmospheric carbon dioxide. For titrations potentiometer Radiometer PHM 64 and the glass electrode G-222B were employed.

The limiting viscosity number $[\eta]$ of solutions of 2,3-dicarboxy derivatives of sodium pectate was determined by means of Ubbelohde viscometer in a mixture of 0.15 mol dm^{-3} NaCl—0.005 mol dm^{-3} sodium oxalate at 25.0 °C.

The IR spectra of 2,3-dicarboxy derivatives of pectic acid were measured in KBr pellets; 0.7 to 2.3 mg of the sample (depending on the intensity of bands) were dissolved together with 200 mg of KBr in 1 cm^3 water, the solution was freeze-dried, the sample transferred into a special press, vacuum dried at 60—70 °C for 3 h and pressed under vacuum at the same temperature. This procedure enabled to remove the maximum of water from discs. The IR spectra were recorded with Perkin—Elmer 577 and UR-20 (Zeiss, Jena) spectrophotometers. The absorption associated with the small amount of water in the particular preparations was compensated by another pellet prepared from the freeze-dried KBr in an analogous way.

Results and discussion

Characterization of 2,3-dicarboxy derivatives of pectic acid

2,3-Dicarboxy derivatives of sodium pectates, needed for IR spectral investigation, were prepared by a two-step oxidation of potassium pectate. The first

oxidation step using $NaIO_4$ afforded 2,3-dialdehyde derivatives of pectic acid, in the second step, using $NaClO_2$, the aldehyde groups were oxidized to carboxyls. The required oxidation degree was achieved by appropriately graduated amounts of oxidation reagents and by a various oxidation time.

The periodate oxidation of pectic acid does not lead to a total oxidation of D-galacturonic acid units to the corresponding dialdehyde. The reaction stops after consumption of 0.64 ± 0.01 mol of $NaIO_4$ per one internal uronic acid unit in the D-galacturonan chain [7]. When interpreting this finding in theoretical terms, the author suggested a random oxidation of uronic acid units in the macromolecule; the reaction is gradually inhibited by unidirectional formation of a six-membered hemiacetal between the C-2 aldehyde group of the oxidized unit and the C-3 hydroxyl group of the not oxidized D-galacturonic acid unit. This limit dialdehyde corresponds, after transformation of aldehyde groups to carboxylic ones, to a 2,3-dicarboxy derivative of D-galacturonan (Na-salt) containing maximally 9.29 mmol (COONa) per 1 g of dry material in the preparation.

The oxidation degree *DO* of substances under investigation expressing the ratio of oxidized D-galacturonic acid units to the number of all units varied within 0.0 to 0.5. Fig. 1 depicts the segment of the partially oxidized macromolecule of D-galacturonan with the structural unit of D-galacturonic acid (*A*) and its 2,3-dicarboxy derivative (*B*) (D-galacturonan forms the main chain of the pectin molecule).

The starting potassium pectate macromolecule contained, in addition to the predominant content of potassium D-galacturonan (85 %), neutral saccharides (15 %, short side chains), which also participate in the periodate oxidation and oxidation with $NaClO_2$ to yield further carboxyl groups. The oxidation degree of the appropriate D-galacturonic acid unit cannot be, therefore, exactly enumerated from the total content of carboxyl groups. The oxidation degree of pectic acid is

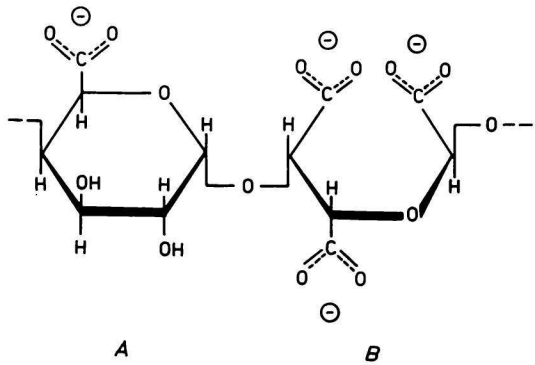

Fig. 1. Segment of partially oxidized D-galacturonan molecule (Na-salt).
A. D-Galacturonic acid unit; *B*. unit of 2,3-dicarboxy derivative of D-galacturonic acid.

considered due to this fact, only according to the total content of carboxyl groups in the preparation.

Content of carboxyl groups in samples of oxidized pectic acid is shown in Table 1 (samples *1* to *7*, series *a*). Since these preparations could contain a small amount of lactones, another series of preparations was prepared from these samples in which the lactones were removed by treatment with alkali (Table 1, samples *1* to *7*, series *b*). The acido- and alkalimetric analysis evidenced that during this preparation no relactonization took place, when very diluted solutions were percolated through a strong cation exchanger-packed column. The high selectivity of the starting preparations for Ca^{2+} ions in the exchange of $Ca^{2+} \rightarrow 2K^+$, described in the preceding paper [3], did not change by the action of sodium hydroxide under the given experimental conditions. Samples *1b*—*7b* revealed a little greater content of carboxyl groups than the starting samples *1a*—*7a*; this corresponds to the presence of 3 to 10 % of the lactone, in average approximately 6 %.

Table 1

Characterization of 2,3-dicarboxy derivatives of pectic acid (Na-salt)

Sample*	Series of measurements		$[\eta]/(cm^3\ g^{-1})$
	a (COONa)/mmol g^{-1}	*b* (COONa)/mmol g^{-1}	
1	4.80 ± 0.06	5.14 ± 0.09	31
2	5.22 ± 0.01	5.47 ± 0.05	27
3	5.48 ± 0.03	6.08 ± 0.08	19
4	6.76 ± 0.05	7.30 ± 0.02	14
5	7.38 ± 0.04	7.56 ± 0.04	—
6	7.11 ± 0.02	7.59 ± 0.02	9
7	8.20 ± 0.02	8.46 ± 0.04	5

* The starting sample: 4.23 ± 0.01 (COONa)/mmol g^{-1}, $[\eta] = 133\ cm^3\ g^{-1}$.

Oxidation of pectic acid is accompanied by a considerable decomposition of the macromolecule. The limiting viscosity number $[\eta]$, as the measure of relative molecular mass of the investigated preparations, decreased from the starting value $[\eta] = 133\ cm^3\ g^{-1}$ as determined in solutions of unoxidized potassium pectate, down to $[\eta] = 5\ cm^3\ g^{-1}$ for the sample with the highest oxidation degree. In spite of this destruction of the macromolecule all preparations showed a high selectivity towards Ca^{2+} ions in the cation-exchange reaction $Ca^{2+} \rightarrow 2K^+$ This cation-exchange selectivity noticeably raised with the increasing oxidation degree in full agreement with the increasing linear charge density of the macromolecule (for detail see [3]).

IR spectra of 2,3-dicarboxy derivatives of pectic acid of various oxidation degree

The IR spectra of sodium salts of 2,3-dicarboxy derivatives of pectic acid were studied in two series of samples. The first series *a* (Fig. 2) consisted of original samples of oxidized pectic acid; these contained a small portion of lactones amounting to 3—10 %. The second series *b* (Fig. 3) comprised the same

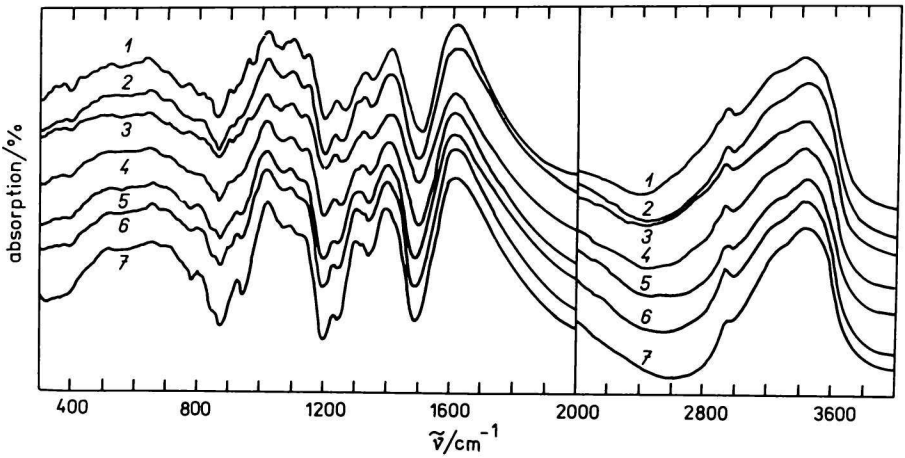

Fig. 2. Spectra of 2,3-dicarboxy derivatives of pectic acid of various oxidation degree. Series *a*. The content of carboxyl groups of the respective samples is listed in Table 1.

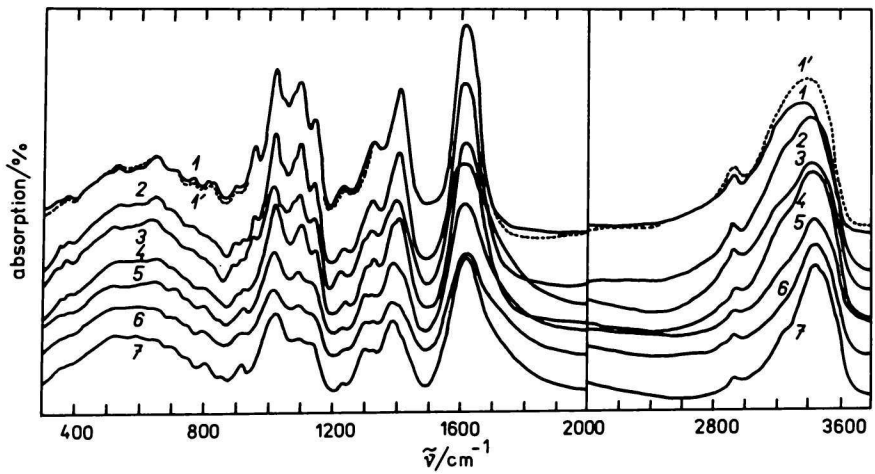

Fig. 3. Spectra of 2,3-dicarboxy derivatives of pectic acid of various oxidation degree. Series *b*. The content of carboxyl groups of the respective samples is listed in Table 1.

preparations without lactones, which were removed by treatment with sodium hydroxide.

Total removal of water from the freeze-dried preparations with KBr was not fully achieved although they were dried under reduced pressure at 60—70 °C. This follows from comparison of spectra of the same disc (sample *1b*) determined with both a KBr plate ("window") in the compensation beam (Fig. 3, dotted curve *1'*), and the KBr disc prepared from the freeze-dried KBr in the same way as with the investigated samples (Fig. 3, full curve *1*). As evident from the ν(OH) absorption (3400 cm^{-1}), the band intensity is higher when the KBr plate is used for compensation. The KBr plate does not contain water and therefore, the spectrum of water sorbed by the preparation is not compensated. The ν(OH) band undergoes alteration with the content of water in the KBr disc, which could not be, however, checked under the experimental conditions. On the other hand, no substantial changes in the remaining region of spectrum were observed when using various discs of KBr in the compensation beam so that the interpretation of spectra was not interfered. Small changes in the band intensities were seen at a wavenumber less than 900 cm^{-1}; these do not, however, change the over-all character of the spectrum, neither the ratio of band intensities of compounds tested.

Spectra plotted in Figs. 2 and 3 document a continuous change with the progressing oxidation of pectic acid. These changes are the same for series *a* and *b*. The spectrum of compound *1* of series *b* (Fig. 3) is virtually identical with that of the unoxidized sodium pectate. (Spectra of pectic substances were already characterized in detail and interpreted in the monograph [4].)

The band wavenumbers of both series of compounds are consistent in the 300—2000 cm^{-1} range, even though spectra of series *a* displayed less sharp peaks, the bands are broader especially for $\nu_{as}(COO^-)$ at about 1610 cm^{-1}. Differences between spectra of both series are greater in the 2000—3000 cm^{-1} region, as *e.g.* reveal the corresponding samples *1a* and *1b*.

Spectra corresponding to the lowest and the highest oxidation degrees of pectic acid (samples *1* and *7*) are presented in Fig. 4. To make comparison of spectra of the respective series more evident, the curves were superimposed. Since various amounts of preparations were used for the preparation of discs (0.7—2.3 mg) with the aim to obtain spectra of an optimum absorption in the band maximum, only changes in the wavenumber and in relative intensities of bands could be considered. The amount of the respective preparation depended on the change of mean molecular mass of the saccharide unit after partial oxidation of pectic acid, on the different content of water in the freeze-dried preparation, on the content of hydrophilic groups (COO^-Na^+), as well as on the degree of cleavage of the pyran ring in the oxidation process. The greatest change in the IR spectrum was found at 1000—1150 cm^{-1}, where intense bands of CC and CO vibrations of pyran ring, characteristic of cyclic saccharides [8] are located. In this region not only the

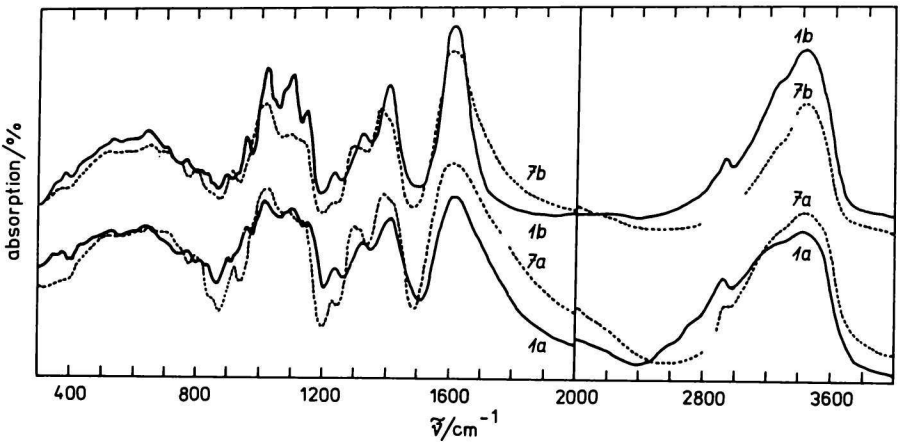

Fig. 4. Comparison of spectra of 2,3-dicarboxy derivatives of pectic acid of the lowest and highest oxidation degrees.
1a, *7a* (series *a*) (4.80 and 8.20 mmol (COONa) g^{-1}); *1b*, *7b* (series *b*) (5.14 and 8.46 mmol (COONa) g^{-1}).

change in band locations, but also in intensity was observed. These changes in the spectra indicate that the oxidation process caused a noticeable change in the pyran ring, in other words its opening.

Oxidation of pectic acid is accompanied by cleavage of pyran rings between C-2 and C-3 and formation of further (COO^-Na^+) groups. Similar, but more complicated changes were encountered when oxidizing the minor component of the pectin molecule, *i.e.* at oxidation of neutral saccharides in side chains in a separate way according to the kind of saccharides and their binding in the macromolecule. The spectra were interpreted paying attention to changes in the primary structure associated first of all with the oxidation of D-galacturonan.

Irrespective of difficult orientation in the ν(OH) vibration region of the spectrum, due to overlapping with the band belonging to water, one can make certain conclusions, especially on the basis of disappearance of weak bands in the 2400—2800 cm^{-1} region of the oxidized samples (Fig. 4). This phenomenon confirms the presumption following from the already reported [4] investigation of IR spectra of deuterated derivatives of pectic acid, namely that these bands correspond to vibrations of hydroxyl groups at C-2 and C-3 involved in hydrogen bonds. Spectrum of methyl pectate revealed these bands at a considerably lower intensity than found with potassium pectate [4]. This can be explained by a greater influence of negatively charged carboxyl groups COO^- upon the system of hydrogen bonds than with an esterified carboxyl group. These bands cannot be identified in the spectrum of pectic acid due to overlapping of bands by an intense ν(OH) band of carboxyl groups forming dimers.

Since samples of series *b* do not contain lactones, they can be considered more homogeneous and their spectra form the basis for our interpretation of results, although no bands characteristic of lactones were identified in series *a*.

Comparison of spectra of compounds *1b* and *7b* (Fig. 4) displays a change in the COO^- group region resulting from oxidation. The band $\nu_{as}(COO^-)$ at 1608 cm^{-1} gets substantially broader, especially towards higher wavenumbers, and becomes asymmetric. Nevertheless, due to the interference of water absorbed, the asymmetry of the $\nu_{as}(COO^-)$ cannot even serve as an evidence for the heterogeneity of carboxyl groups.

The $\nu_s(COO^-)$ vibrational bands are accompanied by far more substantial changes. The increasing degree of oxidation is associated with appearance and raise of $\nu_s(COO^-)$ band at 1380 cm^{-1} in addition to that at 1408 cm^{-1} (Fig. 4). This proves the occurrence of new carboxyl groups differing from those of C-6. The dependence between $\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$ of carboxyl groups and the covalency of bond of metal cations to these groups [9] entitles us to suppose that the bond of metal cations with newly formed carboxyl groups reveals a more covalent character. This presumption is favoured by our results obtained when studying binding of various cations to 2,3-dicarboxy derivatives of pectic acid, starch, and amylose [3, 10]. The couple of carboxyl groups comprising C-2 and C-3 atoms of the oxidized D-galacturonic acid unit in molecules of pectin [3] and 2,3-dicarboxy derivatives of starch and amylose [10] binds the Ca^{2+} far stronger than encountered with C-6 carboxyl groups of pectin, where an intramolecular bond is involved; concretization of the bond type is being studied.

The stable band at 1325 cm^{-1}, independent of the state of carboxyl group, also changes. The percent distribution of potential energy was calculated according to [11, 12]

$$O(C\text{-}4)H(29)O(C\text{-}5)H(13)(C\text{-}1)(C\text{-}2)H(10)(C\text{-}3)(C\text{-}4)H(7)$$

This documents the main contribution of bending vibrations CCH and OCH of the pyran ring to the distribution of potential energy. As seen in the spectrum (Fig. 4), changes of the ring give rise to a new band at 1295 cm^{-1}, which could be anticipated in connection with the bond cleavage between C-2 and C-3.

Another noticeable change of the spectrum was observed in the 1000—1200 cm^{-1} range. The main contribution for distribution of potential energy of the corresponding vibrations is due to stretching vibrations of CC, CO (considered must be also those of (C-2)O, (C-3)O, (C-5)O, and CO of the glycosidic bond). Cleavage of the bond between C-2 and C-3 and formation of further two COO^-Na^+ groups has to lead to fundamental changes of stretching vibrations; this is, however, seen in the spectrum.

Disappearance of the band of medium intensity at 963 cm^{-1} is associated with both the energy levels change of CO and CC vibrations of the pyran ring, and the

decrease of OH groups. As it follows from calculation, a band with potential energy distribution should appear in this region

$$(C\text{-}5)O(28)(C\text{-}2)(C\text{-}3)(17)(C\text{-}5)(C\text{-}6)(9)(C\text{-}1)(O\text{-}5)(8)(C\text{-}6) = O(6)$$

In this, as well as in the preceding description of potential energy distribution, only vibrations with a greater contribution of energy are presented. The remaining energy of vibrations is distributed over further atoms of the pyran ring, but each particular vibration disposes of even smaller energy value.

This band in deuterated derivatives, irrespective of the state of carboxyl groups disappears. This evidences the substantial contribution of hydroxyl groups vibration to the distribution of potential energy; however, this cannot be concluded from the calculation. This band already disappears in the spectrum of sample *4b* (Fig. 3), which is on the other hand in the 1000—1200 cm^{-1} region still consistent with that of sodium pectate. There is a quite insufficient amount of oxidized D-galacturonic acid units in the macromolecule chain to change completely the character of the spectrum indicative of CC and CO vibrations; the band at 963 cm^{-1} is already absent. The soon disappearance of this band, in comparison with the decrease of hydroxyl groups, can be explained by the change of the system of hydrogen bonds connected with the occurrence of new carboxyl groups. Intensity of this band diminishes at low oxidation degrees due to the decrease of OH groups; an acute rebuilding of H-bonds takes place at an already critical oxidation degree, which results even in disappearance of this band.

A change of the spectrum also can be seen in the range of wavenumbers below 900 cm^{-1}. As indicated by calculations, CCO, COC, and τ(OH) vibrations are here responsible for a substantial contribution to distribution of potential energy. It must be considered that spectra in this range will also be affected by changes in conformation of polymeric chains and monomeric structural units. Taking the complexity of the whole system of vibrations into account, it is impossible to assign each spectral band to the particular group in compounds under investigation.

Acknowledgements. Our thanks are due to A. Fekete for his experimental assistance.

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Translated by Z. Votický