

# Infrared spectra of the acetyl derivatives of pectin. I. Potassium salts of the citrus pectic acid with different degrees of acetylation

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Some acetyl derivatives of pectic acid with the degree of acetylation  $a = 0-1.81$  and a very low degree of esterification of the carboxyl groups by methanol ( $e \leq 0.04$ ) were prepared and characterized. The infrared spectra of the potassium salts of these substances obtained with KBr pellets were interpreted. The degree of acetylation of pectate could be easily determined by means of the relative absorbance  $dA$  expressed as a ratio of the absorbances  $A_1/A_2$  determined in the region  $\nu(\text{C}=\text{O})$  of acetyl group ( $A_1$ ;  $1738-1750 \text{ cm}^{-1}$ ) and in the region  $\nu_{\text{as}}(\text{COO}^-)$  of dissociated carboxyl group ( $A_2$ ;  $1615-1630 \text{ cm}^{-1}$ ). The band  $\nu_{\text{as}}(\text{COO}^-)$  was used as an internal standard. A quadratic equation expressing the relationship between the degree of acetylation of pectate ( $a$ ) and the relative absorbance ( $dA$ ) at a known low degree of esterification of the carboxyl groups by methanol was derived.

Приготовлены и охарактеризованы ацетилпроизводные пектовой кислоты с степенью ацетилирования  $a = 0-1,81$  и с очень низкой степенью эстерификации карбоксильных групп метиловым спиртом ( $e \leq 0,04$ ). Интерпретированы инфракрасные спектры солей калия этих веществ, измерены в КВг таблетках. Степень ацетилирования пектата хорошо удалось определить с помощью относительной оптической плотности  $dA$ , выраженной отношением оптических плотностей  $A_1/A_2$ , определенных в области  $\nu(\text{C}=\text{O})$  ацетильной группы ( $A_1$ ;  $1738-1750 \text{ см}^{-1}$ ) и  $\nu_{\text{ас}}(\text{COO}^-)$  диссоциированной карбоксильной группы ( $A_2$ ;  $1615-1630 \text{ см}^{-1}$ ). Полоса  $\nu_{\text{ас}}(\text{COO}^-)$  применилась в качестве внутреннего стандарта. Выведено квадратическое уравнение, выражающее отношение между степенью ацетилирования пектата ( $a$ ) и относительной оптической плотностью ( $dA$ ) при знакомой низкой степени эстерификации карбоксильных групп метиловым спиртом.

The acetylation of the hydroxyl groups of pectin produces considerable change in its properties. For instance, it significantly influences the gel-forming properties of

pectin, the action of pectolytic enzymes on these substrates [1] as well as the ability of pectin to bind metal cations [2, 3].

On the basis of infrared spectra some useful pieces of knowledge about the change in the structure of pectin molecule and the system of hydrogen bonds in the process of the acetylation of hydroxyl groups can be obtained. Though the infrared spectra of the acetates of mono- and oligosaccharides [4, 5] and cellulose [6] have been sufficiently studied, we do not find analogous papers concerned with the acetyl derivatives of pectin. Therefore the aim of this study was to investigate the change in the infrared spectra of pectin as a function of its degree of acetylation.

## Experimental

Commercial citrus pectin, type B, Rapid Set (Københavns Pektinfabrik, Denmark) was used for the preparation of the acetyl derivatives of pectic acid. Pectic acid was prepared by moderate alkaline deesterification in 60% ethanol and purified as described earlier [7]. The acetylation of pectic acid was carried out by using the acetic anhydride in a mixture of formamide and pyridine according to *Carson and Maclay* [8]. The potassium salts of the acetyl derivatives of pectic acid were prepared by neutralization in heterogeneous phase, *i.e.* washing the suspension of acetyl derivatives in 60% ethanol with a dilute ethanolic (60%) solution of potassium acetate the pH of which was adjusted to the value 6.5—7.0. Methyl pectate was prepared by the reaction of pectin with diazomethane [9].

The methods used for the analysis of pectin and pectic acid (determination of the degree of esterification of carboxyl groups by methanol, the content of polygalacturonic acid in dry matter, the viscometric average molecular weight  $\bar{M}_v$ , and the content of neutral saccharides in the preparation) were described earlier [7].

The content of acetyl groups was estimated by distillation method. The acetic acid released was determined alkalimetrically by potentiometric titration [3].

The infrared spectra were measured in the region of 400—4000  $\text{cm}^{-1}$  by using KBr pellets as described earlier [10]. Instruments Perkin—Elmer-577 and UR-20 were used. The absorbances of the bands were determined by the base-line method. The base line was drawn through the section of absorption curve in the region of 1950—1800  $\text{cm}^{-1}$  parallel with the abscissa.

## Results and discussion

Pectic acid used as starting material for the preparation of acetyl derivatives contained 90% of polygalacturonic acid and 10% of neutral saccharides (D-galactose, L-arabinose, D-glucose, D-xylose, and L-rhamnose in molar ratios 9:3:2:1:0.5) in dry matter. Its degree of esterification by methanol  $E$  was 4%, average molecular weight  $\bar{M}_n$  16 500 and content of sulfate ash 0.11%.

The characteristics of the prepared acetyl derivatives of pectic acid are summari-

zed in Table 1. The degree of acetylation  $a$  means the ratio of acetyl and carboxyl groups ( $-\text{OCOCH}_3/-\text{COOH}$ ) in the preparations of pectin\* while the degree of esterification of pectic acid by methanol  $e$  is the ratio of esterified carboxyl groups to their total concentration. For the degree of esterification  $E$  expressed in % it holds:  $E = 100 e$ .

Table 1

Characteristics of the acetyl derivatives of potassium pectate

Sample	Degree of acetylation $a$	Degree of esterification $e$	$\nu_{\text{as}}(\text{COO}^-)$ $\text{cm}^{-1}$	$\nu(\text{C}=\text{O})_{\text{a}}$ $\text{cm}^{-1}$	$D/B$
1	$1.81 \pm 0.02$	0.04	1630	1750	0.51
2	$1.38 \pm 0.01$	0.04	1625	1744	0.59
3	$1.14 \pm 0.06$	0.04	1623	1742	0.62
4	$0.73 \pm 0.03$	0.00	1620	1740	0.69
5	$0.35 \pm 0.03$	0.00	1618	1738	0.71
6	0.00	0.04	1615		

Since the acetyl derivatives of pectic acid did not form any films with suited properties, the KBr pellets had to be used for the study of infrared spectra. As obvious from Fig. 1, the spectra of a high-acetylated preparation (curve 2;  $a = 1.81$ ) and nonacetylated potassium pectate (curve 1;  $a = 0.00$ ) differ significantly from each other and are not conform even with the spectrum of the fully esterified pectin, *i.e.* methyl pectate (curve 3).

During the acetylation of potassium pectate (Fig. 2) a substantial change in spectra takes place, especially the intensity of the band  $\nu(\text{OH})$  in the region of  $3440 \text{ cm}^{-1}$  decreases. However, we did not succeed in finding a correlation between the degree of acetylation and the intensity of this band owing to the superimposition of the band  $\nu(\text{H}_2\text{O})$  and the change produced by pressing pectin with KBr.

The absorption bands characteristic of the acetyl group are as follows:  $\nu(\text{C}=\text{O})_{\text{a}}$  in the region of  $1745 \text{ cm}^{-1}$ ,  $\delta_{\text{s}}(\text{CH}_3)_{\text{a}}$  in the region of  $1380 \text{ cm}^{-1}$ , the intensity of which is for the acetyl group higher than the intensity of the band  $\delta_{\text{as}}(\text{CH}_3)_{\text{a}}$  at  $1440 \text{ cm}^{-1}$ , in contrast to the methyl groups of the methyl ester of carboxyl group. (Index "a" denotes that the vibration is to be assigned to the acetyl group and index

\* The degree of acetylation of the structural units of D-galacturonic acid themselves in a molecule of pectin is approximately by 11% lower than the values  $a$  quoted in Table 1 because the hydroxyl groups of neutral saccharidic units in the molecule are also substituted by acetylation. (For more details see [3].)

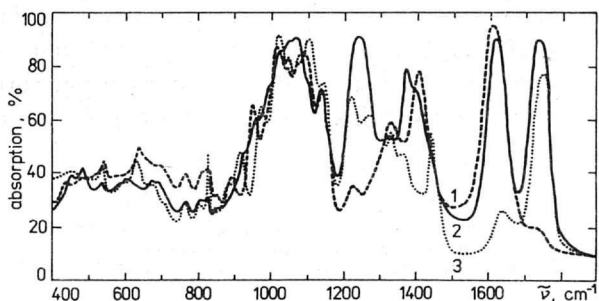


Fig. 1. Spectra of potassium, methyl, and acetyl pectate with a high degree of acetylation.  
 1. Potassium pectate ( $a = 0.00, e = 0.04$ ); 2. high-acetylated potassium pectate ( $a = 1.81, e = 0.04$ );  
 3. methyl pectate ( $a = 0.00, e = 1.00$ ).

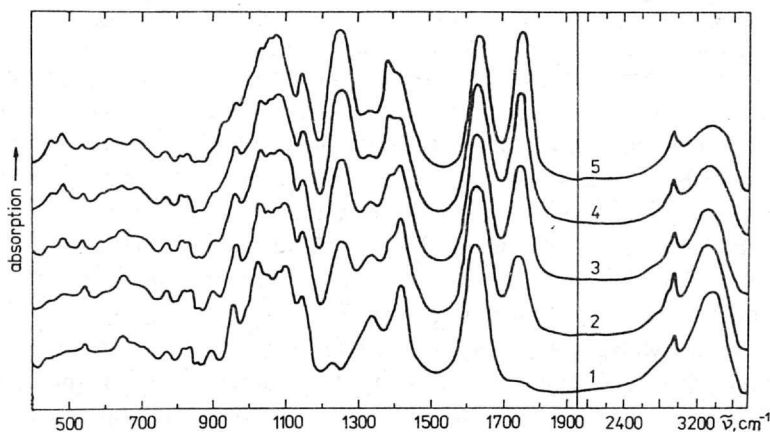


Fig. 2. Spectra of potassium pectates with different degrees of acetylation.  
 1.  $a = 0.00, e = 0.04$ ; 2.  $a = 0.35, e = 0.00$ ; 3.  $a = 0.73, e = 0.00$ ; 4.  $a = 1.14, e = 0.04$ ; 5.  $a = 1.81, e = 0.04$ .

“e” used in subsequent text refers to the methyl ester of carboxyl group.) The band at  $1440 \text{ cm}^{-1}$  is overlapped by an intense band  $\nu_s(\text{COO}^-)$ . The band  $\nu(\text{C—O—C})_a$  in the region of  $1250 \text{ cm}^{-1}$  is much more intense than the corresponding band in the spectrum of methyl pectate.

The band  $\nu_{as}(\text{COO}^-)$  shifts to the region of higher wavenumbers ( $1615 \rightarrow 1630 \text{ cm}^{-1}$ ) with increasing degree of acetylation, which indicates the existence of a hydrogen bond between the ionized carboxyl group and the hydroxyl group in the nonacetylated pectate.

The acetylation of hydroxyl groups of pectin also results in a conspicuous change in the region of wavenumbers under  $1100\text{ cm}^{-1}$  where the spectrum of acetyl derivative is quite different from the spectra of potassium pectate and methyl pectate which are identical. This fact gives evidence that some noncharacteristic complex vibrations of the structural units of pectin exist in the region under  $1100\text{ cm}^{-1}$ , which is in agreement with the conclusions ensuing from a theoretical analysis of cellulose spectra [11]. This change in the region of vibrations of the pyranose rings indicates that the hydrogen bonds affect not only vibrations of the individual groups participating directly in these bonds but also the vibrations of the whole skeleton.

As the acetylated derivatives of pectic acid showed only a very low degree of esterification of the carboxyl groups by methanol ( $e \leq 0.04$ ), we did not take into consideration the change in the band  $\nu(\text{C}=\text{O})_c$  due to the disturbance of the hydrogen bond system and determined the intensities of bands directly. As a matter of fact, this change does not go beyond the experimental errors accompanying the determination of the absorbances  $\nu_{\text{as}}(\text{COO}^-)$  and  $\nu(\text{C}=\text{O})_a$ .

The degree of acetylation of pectic acid with a low degree of esterification of carboxyl groups ( $e$ ) may be determined by means of the ratio of the absorbances found in the region of the maxima of the bands corresponding to the acetyl group  $\nu(\text{C}=\text{O})_a$  and the ionized carboxyl group  $\nu_{\text{as}}(\text{COO}^-)$ . Since the amount of the  $[-\text{COO}^-]$  groups in pectate does not change during the acetylation of pectin, the band  $\nu_{\text{as}}(\text{COO}^-)$  may be used as an internal standard. Notwithstanding, a small shift of this band into the region of higher wavenumbers accompanying the increase in the degree of acetylation causes a certain danger that the intensity of this band might also change. This circumstance was investigated by determining the absorbance ratio of the bands  $\nu(\text{C}=\text{O})_a$  and  $\nu_{\text{as}}(\text{COO}^-)$  for the potassium salts of pectic acid with a varying degree of acetylation.

The absorbance ( $A_1$ ) measured in the region of carbonyl group absorption ( $1738\text{--}1750\text{ cm}^{-1}$ ) is given by the absorption of carbonyls of the acetyl groups (absorbance  $A_a$ ), of the methyl esters of carboxyl groups ( $A_c$ ), and the absorption  $\delta(\text{H}_2\text{O})$  (absorbance  $A'_{\text{H}_2\text{O}}$ ). As shown by the i.r. spectra of the deuterated preparations of pectin the absorption  $\delta(\text{H}_2\text{O})$  at  $1745\text{ cm}^{-1}$  manifests itself only with the salts of pectic acid and pectinic acids.

The absorbance ( $A_2$ ) measured in the region of the ionized carboxyl group ( $1615\text{--}1630\text{ cm}^{-1}$ ) is given by the absorptions  $\nu_{\text{as}}(\text{COO}^-)$  and  $\delta(\text{H}_2\text{O})$  with the corresponding absorbances  $A_B$  and  $A_{\text{H}_2\text{O}}$  (for more detail see [10]).

Therefore it may be written

$$\frac{A_1}{A_2} = dA = \frac{A_a + A_c + A'_{\text{H}_2\text{O}}}{A_B + A_{\text{H}_2\text{O}}} = \frac{D a + C e + k(1-e)}{B(1-e) + m} \quad (1)$$

where  $B$ ,  $D$ ,  $C$ ,  $m$ , and  $k$  are the coefficients of the band absorbances corresponding to  $\nu_{\text{as}}(\text{COO}^-)$ ,  $\nu(\text{C}=\text{O})_{\text{a}}$ ,  $\nu(\text{C}=\text{O})_{\text{c}}$ , and  $\delta(\text{H}_2\text{O})$  at the wavenumbers  $\nu_{\text{as}}(\text{COO}^-)$  and  $\nu(\text{C}=\text{O})_{\text{c}}$ .

The absolute values of these coefficients have not been hitherto determined. On the other hand, their relative values with respect to an arbitrary band of the spectrum can be easily determined. In this case the band  $\nu_{\text{as}}(\text{COO}^-)$  was chosen as the reference band. On dividing the numerator and denominator of eqn (1) by the coefficient  $B$ , we obtain

$$dA = \frac{\frac{D}{B}a + \frac{C}{B}e + \frac{k}{B}(1-e)}{1-e + \frac{m}{B}} \quad (2)$$

Since the intensity of the bands  $\nu(\text{C}=\text{O})_{\text{c}}$  and  $\delta(\text{H}_2\text{O})$  is by decimal order lower than the intensity of the bands  $\nu_{\text{as}}(\text{COO}^-)$  and  $\nu(\text{C}=\text{O})_{\text{a}}$ , we may neglect their change dependent on the degree of acetylation of pectin. For the present, we determined these quantities in a series of pectin samples with a varying degree of esterification of the carboxyl groups by methanol

$$\frac{C}{B} = 0.61, \quad \frac{m}{B} = 0.043, \quad \frac{k}{B} = 0.008$$

The value of  $dA$  was calculated from spectral data. The degree of acetylation ( $a$ ) was determined by the methods described in Experimental.

By inserting all these quantities into eqn (2) we calculated the values of  $D/B$  for a series of the acetyl derivatives of potassium pectate (Table 1). It has appeared that the value of  $D/B$  decreases with increasing degree of acetylation (Fig. 3). At the same time, a small shift of the bands  $\nu_{\text{as}}(\text{COO}^-)$  and  $\nu(\text{C}=\text{O})_{\text{a}}$  into the region of higher wavenumbers appears, which confirms the existence of hydrogen bonds between the carboxyl and hydroxyl groups in the nonacetylated pectate.

By extrapolating the straight line in Fig. 3, the limiting value of  $D/B$  was determined ( $D/B = 0.760$  for  $a \rightarrow 0$ ). The dependence of the quantity  $D/B$  on the degree of acetylation  $a$  is to be expressed in terms of a straight line equation

$$\frac{D}{B} = 0.760 - 0.117 a \quad (3)$$

By substituting all the parameters determined into eqn (2) and rearranging, we obtain

$$a^2 - 6.50 a + \frac{dA(1.043 - e)}{0.117} - 5.15 e - 0.068 = 0 \quad (4)$$

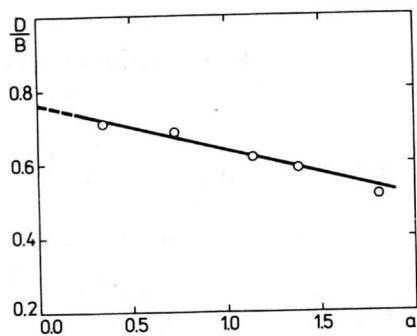


Fig. 3. Dependence of the parameter  $D/B$  on the degree of acetylation of potassium pectate ( $a$ ).

This equation expresses the relationship between the degree of acetylation of pectate ( $a$ ) and the relative absorbance ( $dA$ ) experimentally determined at a low degree of esterification ( $e$ ) of the carboxyl groups of pectin by methanol.

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