Acetyl Pectates and Their Reactivity with Polyvalent Metal Ions[1]

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Acetyl pectates of varying degree of substitution are prepared by the reaction of pectic acid with acetic anhydride in the presence of catalytic amounts of perchloric acid. The pectic acid used has to be activated, *i.e.*, treated with water and then partially dehydrated by washing with a strongly polar solvent. The viscosity of aqueous solutions of the reaction products is found to be higher than that of the starting material indicating that degradation is insignificant. Similar to acetyl alginates, acetyl pectates do not show thickening or gelation with most divalent ions if the degree of substitution is above 1–1.1. This suggests that precipitation of pectate by these ions occurs through chelate formation involving carboxyl groups and pairs of vicinal hydroxyl groups. However, in contrast to alginates, these metal-ion complexes seem to be predominantly intramolecular and not cross linking.

The difficulty with which pectic substances undergo substitution at their hydroxyl groups had been realized decades ago. Attempts to acetylate by conventional methods,[2,3] *i.e.*, reaction with acetic acid or anhydride in the presence of a catalyst and reaction with acetyl chloride in pyridine, etc., were unsuccessful, produced only slight substitution, or, if more rigorous conditions were applied, caused severe degradation. Better results were obtained when nitropectin was used as the starting material.[3]

In 1945, Carson and Maclay[4] succeeded in acetylating pectin, pretreated with pyridine, and pectic acid, after pretreatment with formamide, by the reaction with acetic anhydride in the presence of pyridine. Later, Solms and Deuel[5] repeated the acetylation of pectic acid in order to study gel formation, enzyme activity, and coagulation. They also prepared products with low degrees of substitution by saponification of a highly acetylated pectic acid.

The present article describes the acetylation of pectic acid with acetic anhydride in the presence of catalytic amounts of perchloric acid and the reaction of the resulting products with polyvalent metal ions. This method has the advantage that the free acid is formed which then may be neutralized with any metal hydroxide, carbonate, or bicarbonate and that products of any degree of acetylation (D.A.) can be obtained directly. Another advantage of great importance especially in compatibility studies is the exclusion of possible alkaline degradation or other chemical alterations of the molecule which could occur during saponification with alkali.

For a successful acetylation, it is most important to use pectic acid in an activated form. Recently, it has been shown that the reason for the low reactivity of alginic acid is the formation of hydrogen bonds between hydroxyl groups.[6] Assuming a similar situation in pectic acid which is chemically closely related to alginic acid, the activation may simply consist of reducing the extent of such hydrogen bond formation. This apparently is accomplished by hydrating pectic acid and, in order to avoid larger losses of reagent and excessive development of heat, reducing the water content to a small amount. Direct hydration of pectic acid by treatment with water at room or elevated temperatures is unsuitable. Best results are obtained by precipitating the calcium salt from a solution of sodium pectate and washing the precipitate with dilute hydrochloric acid and then with glacial acetic acid until the residual water content is 5–10%. If the partial dehydration is carried out by washing with acetone or drying in an air stream, the reactivity of the product is greatly reduced, probably because of an uneven distribution of the residual water, excessive drying of the particle surface, and reformation of hydrogen bonds.

The acetyl pectates thus produced have D.A. values of as high as 1.7–1.8. Products with lower D.A. are obtained when the amount of reagent and the reaction time are reduced. However, it is considerably more difficult to obtain acetates of intermediate D.A. than those of low or high D.A., apparently because of a higher rate of reaction at this stage.

Viscosities of 0.052 *N* aqueous solutions of acetyl pectates with varying D.A. from one typical experimental series are shown in Fig. 1. The relatively high viscosities indicate that no degradation has occurred during the acetylation. Only if the temperature exceeds 40–50° may the resulting product be degraded. The temperature is controlled best by adding the catalyst or both the catalyst and the last third of the acetic

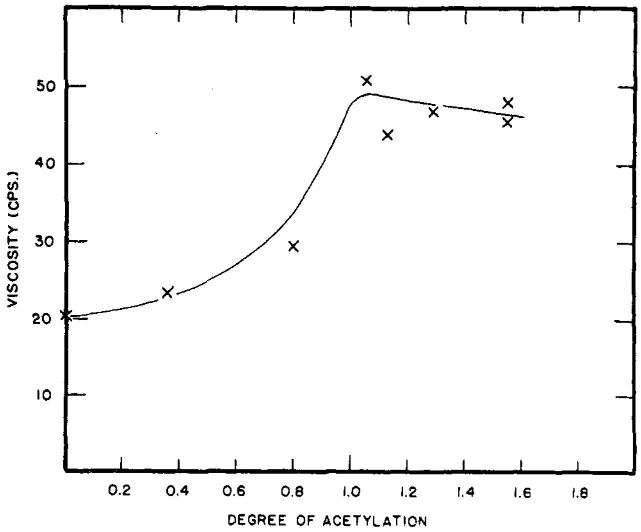

Fig. 1.—Viscosity of 0.052 *N* aqueous solutions *vs.* degree of acetylation.

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[1] Presented before the Division of Carbohydrate Chemistry, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept., 1964.

[2] J. Solms, "Untersuchungen an azetylierten Pektinstoffen," Mitteilung aus dem Agrikulturchemischen Institut der Eidgenössischen Technischen Hochschule, Zürich, 1951, p. 15.

[3] G. Schneider and M. Ziervogel, *Ber.*, **69**, 2530 (1936).

[4] J. F. Carson and W. D. Maclay, *J. Am. Chem. Soc.*, **67**, 787 (1945); **68**, 1015 (1946).

[5] J. Solms and H. Deuel, *Helv. Chim. Acta*, **34**, 2242 (1951).

[6] R. G. Schweiger, *J. Org. Chem.*, **27**, 1786 (1962).

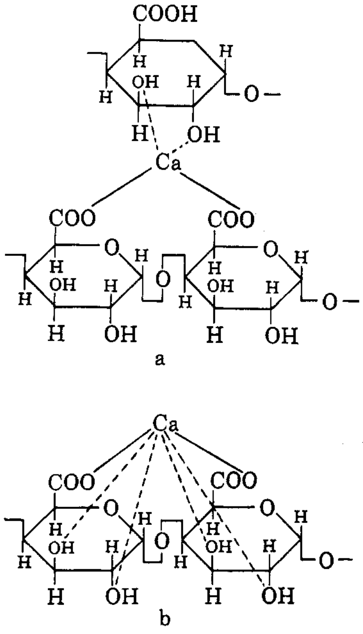

Figure 2.

anhydride slowly in small portions. A substantial drop of the temperature during the reaction should be avoided since it is difficult then to restart the reaction.

Surprisingly, the viscosity at first increases with increasing D.A., exhibits a maximum at a D.A. of about 1, and then decreases slightly again. A viscosity change caused by the introduction of acetyl groups does not explain this phenomenon since a change then should occur consistently in one direction. However, if one assumes the presence of hydrogen bonds in pectic acid, a second, overlapping effect may have to be considered which has been discussed previously in an article on acetyl alginates.[6] If, furthermore, the hydrolysis of glycosidic bonds during the reaction is considered negligible, a curve of the type obtained experimentally in Fig. 1 would indeed be the result.

On testing the compatibility with polyvalent metal ions, another interesting observation was made. Solutions of acetyl pectates with a D.A. of below approximately 1–1.1 formed gels or gelatinous precipitates with calcium or zinc ions; products with a D.A. $\gtrsim 1$ were unaffected (Table I). The critical D.A. at which the reactivity disappears was found to be the same for most other divalent ions as shown in Table II. This clearly indicates that the precipitation of pectate by these ions requires both carboxyl and hydroxyl groups. In the past it has been generally accepted that precipitation occurred through cross linkage by ionic bondings to carboxyl groups only.[7] The fact that only acetyl pectates containing nonacetylated units (D.A. <1) are reactive further suggests that the hydroxyl groups involved have to be vicinal pairs and belong to the same unit. Thus the precipitation reaction may be described as a chelation involving two carboxyl groups and one or two pairs of hydroxyl groups per metal ion. These chelates may be intermolecular (Fig. 2a), *i.e.*, carboxyl and hydroxyl groups belong to different chains, or intramolecular (Fig. 2b), in which case the groups involved in chelation are present in a single residue, adjacent residues, or, generally, in residues of the same chain.

Similar observations were made recently with alginic acid, a glycuronoglycan consisting of β-(1 → 4)-linked D-mannuronic and L-guluronic acid units.[8] The principal structural difference between the units in alginic and pectic acids apparently is the position of the two free hydroxyl groups at C-2 and -3, being *cis* in alginic and *trans* in pectic acid or, if the conformation is considered, axial–equatorial and equatorial–equatorial, respectively, the carboxyl group probably being equatorial in both cases. In spite of this, both substances follow the same chelation principle. Of course, differences are probably noticed in the strength of the chelates which, however, would require additional data for a more accurate estimation. Also, in contrast to acetyl alginates, the fact that acetyl pectates with intermediate D.A. values (even those close to the critical D.A.) usually show graininess when thickened indicates that intramolecular chelate formation (which presumingly reduces or prevents the ability to hydrate, but does not cross link and thus does not increase the size of the polysaccharide molecule) probably predominates.

If the complexes were mainly intermolecular and thus cross linked, as is presumed in alginates, one would expect viscosity increases or soft and smooth gels, but no graininess at the intermediate D.A. values (especially at those close to the critical D.A. because then the increase of the molecular size by cross linkage would be small and below the minimum size required to show reduced solubility indicated by graininess under the conditions applied). The critical D.A. in the acetylation of pectic acid was found to be only slightly above 1 (1 is the theoretical value) indicating that the two reaction steps, uronic acid → monoacetate and monoacetate → diacetate, do not overlap considerably or, in other words, that the corresponding rate constants K_1 and K_2 differ greatly. In contrast, the acetylation of alginic acid produced a higher value for the critical D.A. indicating considerable overlapping and a relatively small difference between K_1 and K_2.

Experimental

Activation of Pectic Acid.—Sodium pectate[9] (100 g.) was dissolved in 6 l. of distilled water, and an aqueous solution of 42 g. of calcium chloride (300 ml.) was added with mixing. The soft precipitate was removed, pressed out, washed with water, then three times with 5–6 l. of 0.1 N hydrochloric acid, and filtered off and pressed out after each washing. In order to remove excess water, the highly swollen product was washed five times with 500-ml. portions of glacial acetic acid and again filtered off and pressed out after each washing. The partially dehydrated material thus obtained was immediately used for the acetylation.

Acetylation.—Activated pectic acid (from 100 g. of sodium pectate), glacial acetic acid (400 ml.), and acetic anhydride (400 ml.) were mixed in a Hobart mixer for 15 min. Then, after adding 200 ml. more of acetic anhydride, about 1.5–2.0 ml. of perchloric acid (70%) was added in 0.1–0.2-ml. portions at such a rate that the temperature of the reaction mixture did not exceed 40–50°. The addition usually was completed after 1–1.5 hr. The end of the reaction usually was indicated by a decrease in temperature which could not be reversed by further addition of perchloric acid. The reaction product was pressed out on a Büchner funnel, mixed thoroughly with water, and filtered off. Washing with water was continued until the wash water had a pH

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(7) Roy L. Whistler, "Industrial Gums," Academic Press, Inc., New York, N. Y., 1959, pp. 409, 416.

(8) R. G. Schweiger, *J. Org. Chem.*, **27**, 1789 (1962).

(9) Purchased from Sunkist Growers, Ontario, Calif.

TABLE I

CONSISTENCY AND/OR VISCOSITY AFTER ADDITION OF CALCIUM CHLORIDE AND ZINC SULFATE

D.A.	Visc., c.p.s.	$CaCl_2$, g.				$ZnSO_4$, g.			
		0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00
0.36	27.5	Gelatinous ppt.				Gelatinous ppt.			
0.70	34.3	Lumpy gel, separates water				Gelatinous ppt.			
0.79	22.6	Grainy gel				4570 Grainy gel	47.5 Thins out		
0.85	21.0	1080 Grainy gel	364 Grainy gel	192	134	4520 Grainy gel	310 Thins out		
0.93	37.4	840 Grainy gel	660 Thins out	590	510	1360 gel	1160	840	680
1.09	24.2	17.5	16.2	16.0	15.5	19.3	17.5	17.5	16.7
1.13	35.0	33.5	33.4	32.3	31.1	34.0	33.1	31.8	31.1
1.29	32.5	27.6	25.3			26.5	26.1		
1.48	25.0	19.0	18.5	18.4	18.5	19.8	18.2	17.8	17.5
1.55	40.3	29.8	29.5			30.7	29.9		
1.72	23.0	16.9	17.1			18.3	16.5		

TABLE II

COMPATIBILITY WITH POLYVALENT METAL IONS[a]

D.A.	0.70	0.79	0.85	0.93	1.09	1.13	1.29	1.48	1.55
Mg^{+2}	−(?)	−	−	−	−	−	−	−	−
Ba^{+2}	++	+	+	+	−	−	−	−	−
Mn^{+2}	+	+	+	+	−	−	−	−	−
Co^{+2}	++	++	++	++	−	−	−	−	−
Ni^{+2}	++	++	++	++	−	−	−	−	−
Cd^{+2}	++	++	++	++	++	++	++	++	++
Cu^{+2}	++	++	++	++	++	++	++	++	++
Pb^{+2}	++	++	++	++	++	++	++	++	++
Fe^{+3}	++	++	++	++	++	++	++	++	++
Cr^{+3}	++	++	++	++	++	++	++	++	++
Al^{+3}	++	++	++	++	++	++	++	++	++
Sn^{+4}	++	++	++	++	++	++	++	++	++

[a] ++, gelatinous precipitate; +, gel or thickening; −, no reaction.

TABLE III

ACETYLATION OF PECTIC ACID

Time, min.	Ac_2O added, ml.	$HClO_4$ added, ml.	Temp., °C.	Sample removed	D.A.
0	260	0.4			
8		0.2	24		
11		0.1	24		
15		0.1			
20		0.1	30		
26		0.1			
33			40		
36				First	0.36
46	100	0.2		Second[a]	0.80
50				Third	1.01
54	100	0.3		Fourth[a]	1.13
57	100	0.3		Fifth[a]	1.29
67	100	0.5		Sixth[a]	1.54
90				Seventh	1.55

[a] Sample was removed before the addition of the acetic anhydride and the catalyst.

of about 5. The acetylated pectic acid was suspended in water, neutralized with ammonium hydroxide, and the thick sirup was poured slowly into acetone with mixing. The soft precipitate was collected, extracted in a Soxhlet with methanol for 20 hr., and dried at 40° in the presence of an air stream. Acetyl determinations carried out as described previously[6] usually indicated a D.A. of 1.6–1.8.

If a series of acetyl pectates with varying D.A. was to be prepared, the initial amount of acetic anhydride was reduced to 250 ml. All other reaction conditions were as described above. More acetic anhydride was added during the reaction. Samples were removed when the desired D.A. was obtained. The approximate D.A. could be estimated by removing small aliquots, blotting them on filter paper, neutralizing as suspension in water with ammonium hydroxide, and testing the consistency of the resulting aqueous solution after the addition of calcium chloride. The course of a typical reaction designed to produce a series of acetyl pectates is shown in Table III.

Products with a D.A. of up to 0.5 were washed with aqueous isopropyl alcohol (the concentration of which was increased from 50 to about 95% with increasing D.A.), those with a D.A. of about 0.5–1.3 required 100% isopropyl alcohol (some benzene was added in the case of the higher D.A. values), and products with a D.A. >1.3 were washed with water. Viscosities of 0.052 *N* aqueous solutions of their ammonium salts (corresponding to 1% ammonium pectate, 1.22% ammonium mono-O-acetyl pectate, or 1.44% ammonium Di-O-acetyl pectate solutions) were measured with a Brookfield Model LVF viscometer at 60 r.p.m. and 25°. In order to exclude any possible influence by traces of calcium ions which still may have been present, 0.2 g. of EDTA was added to each 100-ml. sample before taking the viscosity. The results are shown in Fig. 1.

Compatibility with Polyvalent Metal Ions.—Ammonium acetyl pectate (1 g.) was dissolved in 100 ml. of water. Calcium chloride (1 g.) and, in another experiment, zinc sulfate (1 g.) as concentrated aqueous solutions were added in 0.25-g. portions. After the addition of each portion, the mixture was mechanically stirred or, if necessary, mixed thoroughly with a spatula. The consistency and/or values for the viscosity (Brookfield, 60 r.p.m., 25°) thus obtained are given in Table I for products with varying D.A.

The effect of other metal ions on the gelation or precipitation of acetylated pectic acid was examined in a similar manner by adding one portion of 0.25 g. of the salt/100 ml. of solution (see Table II.) The salts used were as follows: $MgSO_4{\cdot}7H_2O$, $BaCl_2{\cdot}2H_2O$, $MnSO_4{\cdot}H_2O$, $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $Cd(OAc)_2{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, $Pb(OAc)_2{\cdot}3H_2O$, $FeCl_3{\cdot}6H_2O$, $CrCl_2{\cdot}6H_2O$, $AlCl_3{\cdot}6H_2O$, and $SnCl_4$.