Metal Compounds of Pectin

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Received May 16, 1983

Some metal compounds of pectin have been prepared and analysed. The results of various spectroscopic measurements are detailed and discussed. It is suggested that the carboxyl group of the uronic acid residue is bound as a unidentate ligand. Other oxygen donors are either hydroxyl groups from acid residues, or are water molecules.

Introduction

The complexation of metal ions by polysaccharides is of considerable interest in view of the biological role [1–4] that these compounds play, particularly in the availability of nutrients to plants [5, 6], and in the storage of accumulated metals in plant cell walls [7, 8].

The pectins (or pectic substances) are found universally in the primary cell walls and intercellular layers of land plants. They are most abundant in soft tissues such as sugar beet pulp (25%), and apples (15%), but are present in only small proportions in woody tissues. The term pectic substances is used generally to refer to the group of complex plant polysaccharides in which D-galacturonic acid is the principal constituent. The term pectin is used for the gel-forming, water soluble polysaccharides. The D-galacturonic acid units (I) combine with β 1–4 linkages to give polygalacturonic acid chains. Polysaccharides in which a proportion of the galacturonic acid residues are present as methyl esters are designated as pectinic acids; and those devoid of ester groups as pectic acids. Although D-galacturonic acid

is the main sugar constituent of pectic substances, various proportions of other sugars including D-galactose, L-arabinose, D-xylose, L-rhamnose, L-fucose and traces of 2-O-methyl-D-xylose and 2-O-methyl-L-fucose are usually present as constituents.

There have been investigations into the complexing of galacturonic and glucuronic acids and Anthonsen *et al.* [9, 10] have suggested that the metal is chelated to the carboxyl and hydroxyl groups. The formation of such chelates was suggested to be a common reaction of polyuronates or of polysaccharides containing uronic acid residues. Schweiger [11] suggested that pectates form chelates with a number of bivalent metal ions which involve both carboxyl and hydroxyl groups, and suggested that the hydroxyl groups need to be present in vicinal pairs.

Deiana *et al.* [12] have concluded that in the solid (air-dried) state, polygalacturonates of Co(II) and Ni(II) have octahedrally coordinated metal ions, with bidentate carboxylate groups and two axial water molecules. On the basis of its e.s.r. spectrum they have concluded that Cu(II) polygalacturonate is tetragonally distorted.

In this paper we present data on metal pectates and pectinates. Copper galacturonate is included for comparison.

Experimental

Preparation of the Compounds

Compounds were prepared by the methods of Schweiger [11] and Wunsch [13]. The preparations were carried out by adding weighed amounts (1–2 g) of pectin (citrus origin) in 65% aqueous isopropyl alcohol (15 cm^3) to sodium hydroxide (~0.15 g). The suspension was then stirred for 15 minutes until the hydroxide pellets had completely dissolved. Varying concentrations of metal salts in a small volume of 65% isopropyl alcohol were added to the

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TABLE I. Analysis Results for Pectic Type Compounds.

Sample[a]	Formulae	Calculated		Found	
		C%	H%	C%	H%
Aluminum pectinate[b]	$C_{12}H_{14}O_{12}Al \cdot 5H_2O$	30.8	5.1	30.8	5.3
Cobalt pectate	$C_{12}H_{14}O_{12}Co \cdot 4H_2O$	29.9	4.6	29.4	4.9
Cobalt pectinate[b]	$C_{12}H_{14}O_{12}Co \cdot 2H_2O$	32.6	4.6	32.8	4.8
Copper pectate	$C_{12}H_{14}O_{12}Cu \cdot 4H_2O$	29.6	4.5	29.2	4.6
Copper pectinate[b]	$C_{12}H_{14}O_{12}Cu \cdot 4H_2O$	29.6	4.5	29.0	4.4
Copper galacturonate	$C_{12}H_{18}O_{14}Ni \cdot 4H_2O$	27.6	5.0	26.9	4.9
Nickel pectate	$C_{12}H_{14}O_{12}Ni \cdot 5H_2O$	28.8	4.8	28.8	4.9
Nickel pectinate[b]	$C_{12}H_{14}O_{12}Ni \cdot 5H_2O$	28.8	4.8	28.2	5.1
Zinc pectate	$C_{12}H_{14}O_{12}Zn \cdot 4H_2O$	29.5	4.5	29.5	4.8
Zinc pectinate[b]	$C_{12}H_{14}O_{12}Zn \cdot H_2O$	33.2	3.6	33.2	3.9

[a]Air-dried and stored over P_2O_5.   [b]For pectin and pectinates 7% methoxy was included in the calculations.

suspension and the mixture was allowed to equilibrate at room temperature under magnetic stirring. The solid samples were filtered off and washed several times with 65% isopropyl alcohol to remove soluble salts. After the alcohol rinse, the complex was air dried in the funnel, and then kept over phosphorus pentoxide (under vacuum). When dry, the product was weighed, ground using a pestle and mortar and left over phosphorus pentoxide. For metal pectates, pectic acid was used instead of pectin and the method of preparation proceeded as described for the preparation of metal pectinates.

Copper galacturonate was prepared by adding weighed amounts (0.5–1 g) of the acid to ~0.1 M solution (~100 cm^3) of copper sulphate solution and allowing the suspension to equilibrate at room temperature under magnetic stirring. The complex formed was then filtered and washed several times with water. Analysis results are shown in Table I.

Spectroscopic Measurements

Infrared spectra were measured in KBr using a Perkin Elmer 197 spectrophotometer. Electronic spectra of freshly ground samples were measured using a Pye-Unicam SP700 recording spectrophotometer fitted with the SP 735 solid state attachment, and with freshly prepared MgO as reference. Electron spin resonance spectra were obtained on a Decca X-3 spectrometer at 9270.301 MHz, fitted with a proton resonance probe for field determination and using 100 KHz modulation. ^{13}C solid state nuclear magnetic resonance spectra were recorded with a Jeol FX-60 QS spectrometer with magic angle spinning.

TABLE II. Carboxylate Stretching Frequencies (cm^{-1}) in Air-Dried Metal Pectates and Pectinates.

Metal complex	$\nu_a(CO_2)$	$\nu_s(CO_2)$	Δ[a]
Zinc pectate	1610s	1410s	200
Zinc pectinate	1610s	1410s	200
Cobalt pectate	1610s	1410s	200
Cobalt pectinate	1610s	1410s	200
Nickel pectate	1610s	1415s	195
Nickel pectinate	1610s	1415s	195
Copper pectate	1620s	1405s	215
Copper pectinate	1620s	1405s	215

[a]$\Delta = (\nu_a - \nu_s)$.

Results

Infrared Spectra

Infrared spectra of metal pectates and pectinates exhibit two sharp bands in the range 1600–1635 cm^{-1}, assigned respectively to the $\nu_a(CO_2)$ and $\nu_s(CO_2)$ modes of the carboxylate group [14]. The positions of these bands are shown in Table II.

Electronic Spectra

Both the pectate and pectinate complexes of cobalt show bands occurring at 8,000, 14,500 sh, and 18,500 cm^{-1}. Similarly, Ni(II) complexes exhibit absorption bands which indicate octahedral coordination, occurring at 8,000 cm^{-1} in pectate and 8,500 cm^{-1} in pectinate ($^3A_{2g} \rightarrow \ ^3T_{2g}$), a shoulder at 13,500 cm^{-1} in both pectate and pectinate ($^3A_{2g} \rightarrow \ ^3E_g$), band at 14,500 cm^{-1} in pectate and 15,000 cm^{-1} in pectinate ($^3A_{2g} \rightarrow \ ^3T_{1g}(F)$), and 15,000 cm^{-1} in both pectate and pectinate ($^3A_{2g} \rightarrow \ ^3T_{1g}(P)$). The

TABLE III. E.S.R. Parameters of Copper Complexes.

| Complex	State	g_{11} (±0.005)	$g_\perp$ (±0.005)	A_{11} (±5 Gauss)	$A_\perp$ (±5 Gauss)
Copper galacturonate	Air-dried	2.263	2.080	–	–
Copper galacturonate	Solution (pH 5)	2.354	2.063	140	20
Copper pectate	Air-dried	2.380	2.062	120	–
Copper pectate	Solution in NaOH	2.252	2.062	185	30
Copper pectinate	Air-dried	2.375	2.060	130	–
Copper pectinate	Solution in NaOH	2.251	2.062	185	35
Copper polygalacturonate[a]	Air-dried	2.389	2.070	120	–
Copper polygalacturonate[a]	Hydrated	2.390	2.070	122	20

[a]From reference [9].
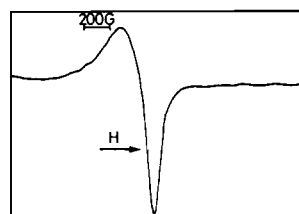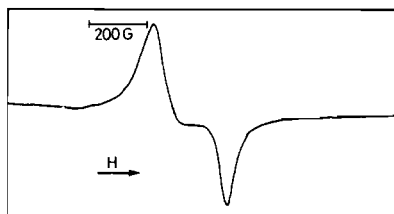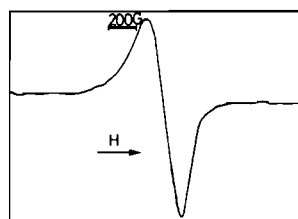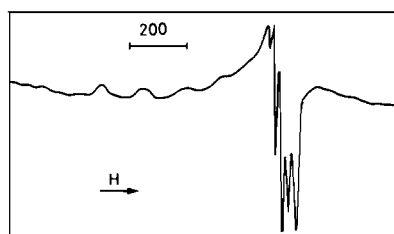
Fig. 1. E.S.R. Spectra of copper galacturonate; (a) top: frozen solution (10^{-3} *M*) in acetate buffer (pH 5); (b) bottom: room temperature, solid state.

Fig. 2. E.S.R. Spectra, room temperature, solid state; (a) top: copper pectate; (b) bottom; copper pectinate.

electronic spectra of copper compounds are characterized by a broad structureless band showing a maximum around 12,500 cm^{-1}.

Electron Spin Resonance Spectra

The room temperature specimen of solid (air dried) copper galacturonate is shown in Fig. 1a, with g_{11} = 2.263 and $g_\perp$ = 2.08. A frozen solution in acetate buffer (pH 5) gave an anisotropic axial spectrum with spectral parameters: g_{11} = 2.354, $g_\perp$ = 2.063; A_{11} = 140, and $A_\perp$ = 20 G. These parameters are characteristic of a copper environment with relatively strong axial distortion [15]. The e.s.r. spectra of air dried copper pectate and copper pectinate, and of solutions in 0.1 *M* sodium hydroxide are shown in Figs. 2 and 3. The spectra are

well resolved and the spectral parameters are detailed in Table III.

Nuclear Magnetic Resonance Spectra

This study was limited to the solid state because of the insolubility of the polymers and their complexes. Some solid state n.m.r. spectra are shown in Fig. 4 and Table IV.

Discussion

The analyses results (Table I) show that there is one metal ion for two galacturonate units. All compounds were air dried and stored over P_2O_5 but even so there appears to be a great variation in the numbers of water molecules present.

TABLE IV. ^{13}N.m.r. spectra of Pectin and Derivatives (Solid State, ppm)

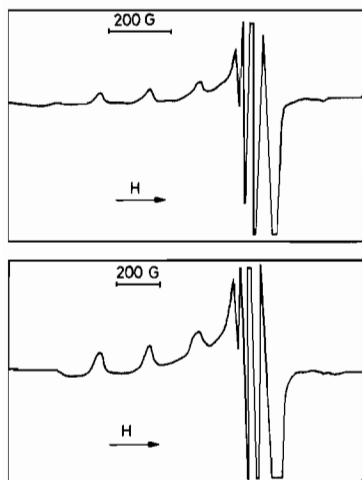|	C-1	C-2	C-3	C-4	C-5	C-6	Me
D-galacturonic acid	94.50	68.68	73.53	73.35	69.74	174.4	–
polygalacturonic acid	101.1	~70	–	83.0	69.7	172.5	–
pectin	101.1	71.4	–	78	69.1	171.7	53.2
Al pectin	101.4	~71	–	~78	–	174.4	~54
Zn pectin	102.2	~72	–	~85	–	174.7	~54
Ni pectin	101.2	~72	–	~81	–	175.5	~54

Fig. 3. E.S.R. Spectra, frozen solution in NaOH (0.1 *M*); (a) top: copper pectinate; (b) bottom: copper pectate.

Infrared measurements: In pectic acid and pectin the carboxyl group absorption appears at 1730 cm^{-1}, on complexation this disappears and is replaced by the antisymmetrical (ν_a) and symmetrical (ν_s) vibrations of the CO_2 group [15, 16]. In a series of metal compounds with a carboxylate ligand, in which there is increasing metal-oxygen interactions, ν_s decreases and ν_a increases, while Δ, the separation between the two, increases.

Robertson and Uttley [17] have characterised unidentate and bidentate modes of bonding by a consideration of the CO_2 group frequencies. They found that $\nu_a(CO_2)$ and Δ values are closer to those in the free ion for a chelated four membered ring type of coordination, while Δ values for unidentate coordination are much larger, and ν_a bands appear at higher frequencies (Table V).
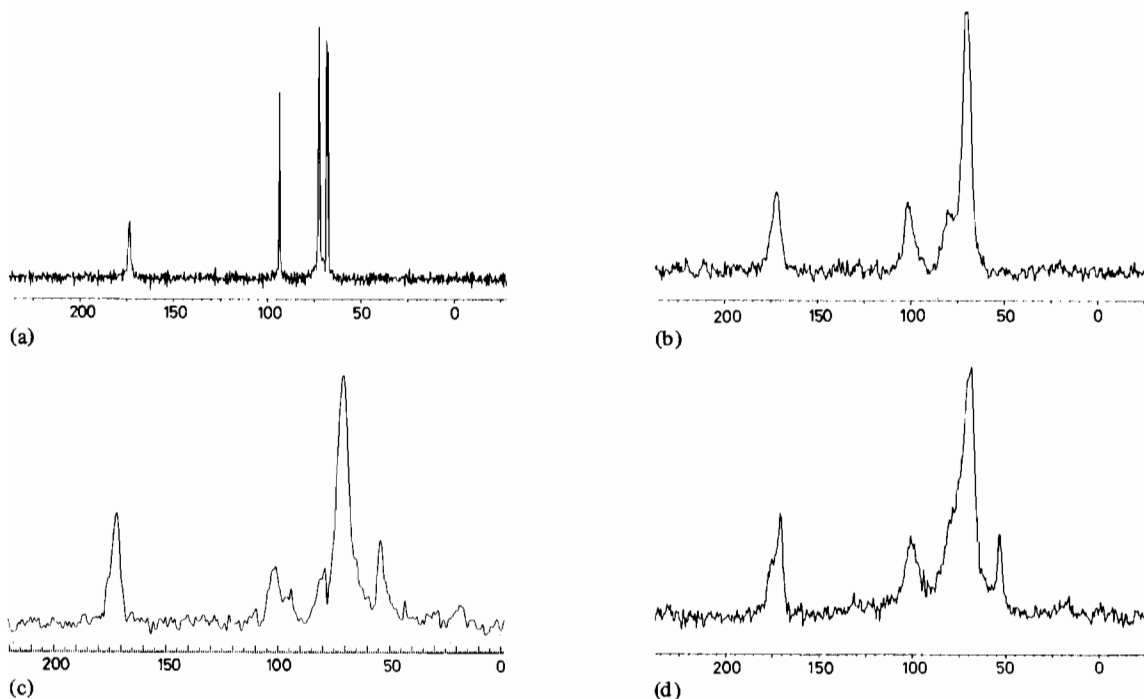

Fig. 4. Solid state ^{13}C N.M.R. spectra; (a) D-galacturonic acid; (b) polygalacturonic acid; (c) pectin; (d) nickel pectinate.

TABLE V. Data for CO_2 Stretching Vibrations in Carboxylate Complexes (cm^{-1}).

	$\nu_a(CO_2)$	$\nu_s(CO_2)$	Δ
chelates (4m ring)	1490–1540	1400–1470	40–120
unidentate	1580–1650	1310–1390	210–170

Table II shows that all the bands come within the range for unidentate coordination. Thus the bonding in compounds of bivalent Co, Ni, Cu and Zn are probably similar. The evidence suggests that bidentate carboxylate bonding, as suggested by Deiana *et al.* [12] for the polygalacturonates, does not occur.

Some e.s.r. parameters for copper compounds are shown in Table III. The result suggest that the compounds have similar structures with respect to the copper. In frozen solutions the spectra are characteristic of a copper environment with a relatively strong axial component [15, 18, 19].

^{13}C n.m.r. study: The ^{13}C n.m.r. study was limited to the solid state, since the polymeric substrates are insoluble. The band assignments are tentative and derived from other studies in the literature [20–22]. The formation of one molecule of a disaccharide from two molecules of a sugar results in a marked deshielding of the C-4 of one molecule as well as the C-1 of the other [22]. Table IV shows that on going from galacturonic acid to polygalacturonic (which involves a β 1–4 link), C-1 is shifted by +6.6 ppm and C-4 is shifted by +9.65 ppm. These shifts are similar to those in β-cellobiose [22], where C- is the glucopyranosyl moiety was shifted by 6.49 ppm, and C-4 in the glycopyranose ring was shifted by 10.51 ppm. The resonances in pectin and metal-pectin compounds are broad and unresolved, however, some trends can be seen. The C-6 carbon is slightly deshielded in the metal compounds and the methylation can be seen by the appearance of the methyl carbon resonance at ~54 ppm.

Kohn [23] and Lakatos [24] consider pectate complexes to be outer sphere. Our results indicate that the interactions do not produce strong ligand fields. In a consideration of metal compounds of alginic acid, Schweiger [11] suggested that in the calcium compound, the metal ion bonds to carboxyl groups, probably on neighbouring units, two further bonds are formed with vicinal OH groups on a third unit. This third unit may be in the same chain, keeping the macromolecule coiled, or may be on another chain forming a cross-linked polymer. Gel-formation would tend to indicate the latter, inter-chain type of bonding. It was suggested that in pectates, the ratio of inter- to intramolecular forms decreases in the order:

$$Cu^{2+}, Ca^{2+} > Mg^{2+} > Ni^{2+}, Sr^{2+} > Mn^{2+}, Co^{2+}, Ba^{2+} > $$
$$> Cd^{2+} > Zn^{2+}.$$
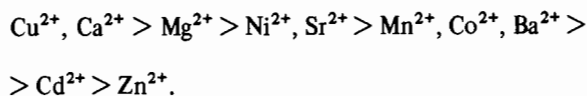
It does not appear that size is of major importance since both zinc and aluminium (effective ionic radii 0.74 Å and 0.535 Å respectively [25]) formed similar compounds. The stereochemistry of Cu^{2+} compounds, where there is tetragonal distortion, would lead to increased formation of intermolecular forms, as the longer axial positions could be occupied by O-donors from other polymer chains. It is also interesting that the numbers of water molecules involved in the pectinates decrease in the order:

$$Cu^{2+}(6H_2O) > Ni^{2+}(5H_2O) > Co^{2+}(2H_2O) > $$
$$> Zn^{2+}(H_2O).$$
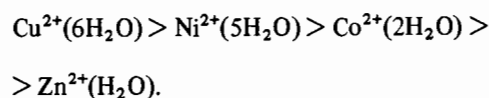
This order agrees with that found from gel-formation. It seems likely that where there is a majority of intermolecular bonding the metal ion, or the polymer, is associated with water molecules, adding to the 'openness' of the 3-dimensional network by increasing the interpolymer distance.

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