BINDING OF CALCIUM, LEAD, AND COPPER(II) CATIONS TO GALACTARIC AND 2,5-FURANDICARBOXYLIC ACIDS AND TO D-GALACTURONIC ACID AND ITS DERIVATIVES

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The binding of Ca^{2+}, Pb^{2+}, and Cu^{2+} ions to galactaric and 2,5-furandicarboxylic acids and of Pb^{2+} and Cu^{2+} ions to D-galacturonic acid, its α-methylglycoside, and (methyl-4-deoxy-α-D--galactopyranosid)uronic acid was evaluated in terms of the activity coefficients of the counter--ions and the degrees of their association with the carboxylate groups of the acids. The activity of Ca^{2+} ions was determined by the metallochromic indicator method with tetramethylmurexide, that of Pb^{2+} and Cu^{2+} ions, with ion specific electrodes. Galactaric acid binds 89·5% Ca^{2+} ions, practically all (99·9%) Pb^{2+} ions and nearly all (98·6%) Cu^{2+} ions, mostly with the formation of insoluble salts. As little as 7·6% Ca^{2+}, 34·0% Pb^{2+}, and 23·9% Cu^{2+} were bound to 2,5-furandicarboxylic acid in the form of soluble complexes, and slightly more, 4·1% Ca^{2+}, 43·9% Pb^{2+}, and 38·0% Cu^{2+}, to D-galacturonic acid. The difference in the degrees of binding to galactaric acid and 2,5-furandicarboxylic acid is due to the different flexibility of their molecular skeletons. The activities of Pb^{2+} and Cu^{2+} ions in solutions of salts of D-galacturonic acid and its derivatives indicate that the OH group at $C_{(4)}$ of D-galacturonic acid takes part in the complexation. For the Pb^{2+} complex, the OH group at $C_{(1)}$ of the unsubstituted acid in the β-anomeric form also seems to participate in the complexation; this is not the case with the similar Cu complex.

Clinical tests of the efficacy of pectin as a prophylactic and remedy in case of lead poisoning have shown that after its peroral application, an increased excretion of lead occurs not only in excrements but also in urine, hence, off the gastrointestinal tract[1,2]. At chronic lead poisoning, pectin brings about a substantial improvement of the conditions of the diseased person's health and even contributes to the elimination of lead deposited in the body. It has been suggested that the ligand for lead in the blood circulation system may be D-galacturonic acid as a degradation product of pectin[2,3]. An increased level of uronic acid in the peripheral blood serum was observed during the study of the hemostatic effect of pectin after its peroral application[4]. In experiments on rats, D-galacturonic acid was proved to be partly resorbable in the small intestine[5]. After intoxication of respiratory organs by mercury vapours, an enhanced excretion of Hg^{2+} ions in urine can be achieved by peroral administration of pectin[6]. According to Sapozhnikova and coworkers[7], however, the prophylactic effect of D-galacturonic acid on lead poisoning has not been proved.

Previously we studied the binding of lead[8], copper, cadmium[9], zinc[10], calcium and strontium[11] to D-galacturonic acid and oligomeric pectin fragments of degrees of polymerization DP = 2–9. While the binding of Ca^{2+}, Sr^{2+}, and Zn^{2+} to monomeric D-galacturonic acid is negligible, the other cations mentioned are bound to it to an appreciable extent in order $Cd^{2+} < Cu^{2+} < Pb^{2+}$.

Other low molecular weight pectin metabolites are galactaric (mucic) and 2,5-furandicarboxylic acids. Galactaric acid is an oxidation product of galactose-containing saccharides (plant gums, pectin), and it has been identified in various fruits[12]. It is formed, *e.g.*, in grapes by oxidation of D-galacturonic acid under the action of enzymes of parasitic moulds[13]. 2,5-Furandicarboxylic acid is a dehydration product of galactaric acid. Its amount in human urine was found to increase 5 to 15 times on a daily administration of 5 g of D-galacturonic acid[14].

Galactaric acid forms very stable complexes with a number of divalent cations. The complex formation with rare earth ions has been studied in detail, *e.g.*, in refs[15,16]. With some divalent cations the acid forms salts which are low soluble in water[17] (10^{-5}–10^{-3} mol l^{-1}). It is used as a chelating agent for Ca^{2+} ions, *e.g.*, as an additive added to alkaline detergents[18], for decalcification of wine[19], as a chromogenic reagent for the spectrophotometric determination of trace amounts of some divalent cations[20], *etc.* With lead[21] and copper(II) (ref.[22]) ions, galactaric acid forms relatively strong complexes in various component ratios. Polarographic evidence was also obtained of the formation of several complex species of 2,5-furandicarboxylate ions with Cu^{2+} cations; the stability constants of the complexes increase markedly with increasing number of acid anions in the complex per Cu^{2+} cation[23].

The present work is concerned with the binding of the essential calcium cations and the toxic lead and copper(II) cations to galactaric and 2,5-furandicarboxylic acids and to D-galacturonic acid (*I*) and its two model derivatives, *viz.* (methyl-α-D-galactopyranosid)uronic acid (*IV*) and (methyl-4-deoxy-α-D-galactopyranosid)-uronic acid (*V*); the purpose of this study was to contribute to the elucidation of the mechanism of elimination of lead from the human body on the peroral administration of pectin.

EXPERIMENTAL

Chemicals

D-Galacturonic acid (*I*) monohydrate, puriss., was a preparation of Fluka (Buchs). Methyl (methyl-α-D-galactopyranosid)uronate (*II*) was prepared by procedure[24], methyl (methyl-4-deoxy-α-D-galactopyranosid)uronate (*III*), by procedure[25]; both were chromatographically (TLC) pure. Galactaric acid was a product of Spolek pro chemickou a hutní výrobu, Ústí nad Labem; its purity was verified by determination of the carboxy group content. 2,5-Furandicarboxylic acid was kindly supplied by E. Körblová, Institute of Organic Chemistry and Biochemistry, Czecho-

slovak Academy of Sciences, Prague; for $C_6H_4O_5$ (156·1) calculated: 46·16% C, 2·58% H; found: 46·17% C, 2·61% H.

The following solutions were used: 0·05M-KOH, carbonate-free; 0·021M-$Ca(OH)_2$ (saturated); 0·01M-$Pb(NO_3)_2$; 0·01M-$Cu(NO_3)_2$; 0·01M and 0·002M-Chelaton IV and $MgCl_2$; tetramethylmurexide[26]; and redistilled water freed from CO_2. All the other chemicals were of reagent grade purity.

Preparation of Solutions of Potassium Salts of Acids Studied

Galactaric acid was dissolved by slow neutralization with 0·05M-KOH to obtain a solution with a COOK group concentration of 5—7 mmol l^{-1}. Lactones, if present in a small quantity in the preparation, were eliminated by the alkaline medium in a hermetically closed vessels (0·01M-KOH, 23—25°C, 18 h). Excess hydroxide was removed by percolation of the solution through a column of Dowex 50 W×2 (H^+) cation exchanger; in this manner, the potassium galactarate was converted to galactaric acid. The eluate was neutralized to the equivalence point with 0·05M-KOH, whereby a solution of the salt was obtained in a known concentration.

2,5-Furandicarboxylic acid was also dissolved by neutralization with 0·05M-KOH to obtain a solution with a COOK group concentration of 5—7 mmol l^{-1} and percolated through a cation exchanger column, and the eluate was neutralized with 0·05M-KOH.

Methyl (methyl-α-D-galactopyranosid)uronate (*II*) and methyl (methyl-4-deoxy-α-D-galactopyranosid)uronate (*III*) were subjected to gentle alkaline deesterification. The amount of 0·05M-KOH requisite for this was established in advance acidimetrically, excess hydroxide being determined by potentiometric titration with 0·01M-HCl. To 0·25—0·30% solution of *II* was very slowly (during 3 h) added the theoretical amount of 0·05M-KOH so that pH did not exceed pH 10·0; a toluene layer protected the solution and titrant from contact with air CO_2. The solution was allowed to stand for 0·5 h and then percolated through a Dowex 50 W×2 (H^+) column. The eluate (solution of the uronic acid derivative) was neutralized to the equivalence point with 0·05M-KOH. For *III*, the same procedure was used, only KOH was added in a 10% excess relative to the theory and the excess was eliminated by means of the cation exchanger (the solution was protected against air CO_2); further proceeded as with *II*.

Determination of Activities of Ca^{2+}, Pb^{2+}, and Cu^{2+} Counter-Ions

The activity of calcium ions was determined in solutions of calcium salts of galactaric and 2,5-furandicarboxylic acids without ionic strength adjustment. Solutions of the two acids with COOH group concentrations about 4 mmol l^{-1} were obtained by percolation of solutions of their potassium salts through a cation exchanger column as above, and neutralized to the equivalence point with calcium hydroxide solution and diluted to $c_{COOCa_{0.5}} = 3{\cdot}00$ mmol l^{-1}. The solution of calcium 2,5-furandicarboxylate was clear, whereas in the galactaric acid solution a precipitate of the calcium salt was observed to form during the neutralization. The resulting solution or suspension were allowed to stand for 18 h. The suspension was centrifuged at 20 000 *g* for 20 min and the total calcium concentration in the supernatant (c_{Ca}) was determined chelatometrically (see later). The calcium ion activity ($a_{Ca^{2+}}$) was determined in the clear solution or supernatant with a metallochromic indicator (tetramethylmurexide) as described previously[26,27]. The starting ionic strength of the solution was $I_0 = 0{\cdot}0045$ mol l^{-1}. A Hilger Uvispec spectrophotometer was used.

The activities of lead and copper ions were determined in solutions of potassium salts of the acids examined, of a final concentration of COOK groups of 3·00 mmol l^{-1}, with additions of lead or copper nitrate precisely equivalent to the carboxy group content ($c_{M^{2+}} = 1{\cdot}500$ mmol.

. l^{-1}); for galactaric acid, only 96% metal ion additions (1·435 mmol l^{-1}) were used in order to prevent any possible contribution of the metal nitrate to the very low concentration (activity) of the cation in the final equilibrium system. The ionic strength was adjusted with 0·1M-KNO_3 to I_0 = 0·01 mol l^{-1}. In the case of galactaric aicd, its lead and copper salts precipitated. The solutions or suspensions were stirred for 2 h and then allowed to stand for 18 h. The clear solutions were used for the activity measurements directly, the suspensions were centrifuged at 20 000 *g* for 20 min and the total lead or copper ion concentrations and activities were determined in the supernatant.

The activities $a_{Pb^{2+}}$ and $a_{Cu^{2+}}$ were determined at 25·0 ± 0·1°C with Crytur 82—17 and 29—17 ion specific electrodes (Monokrystaly, Turnov) as described previously[8,9]; a K-711 saturated calomel electrode with a two-compartment bridge (Radiometer, Copenhagen) served as the reference electrode (the outer compartment of the bridge was filled with 10% KNO_3). A PHM 64 potentiometer (Radiometer, Copenhagen) was used. A G-222B glass electrode and a K-401 calomel electrode (Radiometer, Copenhagen) were employed for the potentiometric titrations.

The total calcium, lead or copper concentrations in the supernatants were determined chelatometrically with a spectrophotometric indication of the end point (interference filters of Zeiss, Jena; Ca, Cu: IF 600 nm, Pb: IF 650 nm). Calcium and copper were determined by direct titration using murexide as the indicator, lead was determined by retitration of excess Chelaton IV with 0·01M or 0·002M-$MgCl_2$ using Eriochrome Black T as the indicator. Corrections found in blank experiments were made in the determination of calcium and lead.

The ionic strength of the equilibrium solution (I) was calculated from the component contents of the starting solution and the metal ion activity determined in the equilibrium solution by the iterative procedure[8].

The degrees of association of the divalent cations with the carboxy groups of the acids were calculated as

$$\beta = (c_{M^{2+}} - [M^{2+}]_f)/c_{M^{2+}} ,$$

where $c_{M^{2+}}$ is the initial concentration of the cation in the solution or suspension and $[M^{2+}]$ is the concentration of the free ions in the equilibrium solution.

RESULTS AND DISCUSSION

Binding of Calcium, Lead, and Copper (II) Ions to Galactaric and 2,5-Furandicarboxylic Acids

The binding of calcium ions to the carboxy groups of the acids was examined without adjusting the ionic strength of the solution by adding supporting electrolyte; the initial ionic strength, corresponding to the $COOCa_{0.5}$ group concentration of 3·00 mmol . . l^{-1}, was I_0 = 0·0045 mol l^{-1}. For the two other metals, the ionic strength was adjusted with KNO_3 to I_0 = 0·01 mol l^{-1}. So low ionic strengths were used with regard to the fact that the counter-ion binding is then stronger than at higher ionic strengths (see later), so that the amount of bound cations can be determined more accurately. The binding of the cations to the carboxy groups, giving rise to undissociated complexes or an insoluble salt, results in a decrease in the ionic strength of the equilibrium solution (Tables I and II).

Galactaric acid possesses acyclic flexible molecules (Fig. 1), owing to which the two carboxy groups can assume an optimum steric arrangement for the binding of divalent cations. 2,5-Furandicarboxylic acid, on the other hand, has a rigid cyclic skeleton and mutually oppositely oriented carboxy groups. As a result, the two acids differ in their ability to bind divalent metal cations.

TABLE I

Binding of calcium, lead, and copper(II) cations to galactaric and 2,5-furandicarboxylic acids

Acid	M^{2+}	c_M mmol l^{-1}	$[M^{2+}]_f$ mmol l^{-1}	$a_{M^{2+}} \cdot 10^3$	$\gamma_{M^{2+}}$	I mol l^{-1}	β
Galactaric	Ca^{2+} [a]	0·181	0·158	0·146 ± 0·003	0·807	0·0005	0·895 ± 0·003
	Pb^{2+} [b]	0·009	0·0015	0·0015 ± 0·0005		0·0055	0·999 ± 0·001
	Cu^{2+} [b]	0·117	0·020	0·015 ± 0·0005	0·128	0·0056	0·986 ± 0·001
2,5-Furan-	Ca^{2+} [a]	1·500	1·386	1·062 ± 0·007	0·708	0·0042	0·076 ± 0·007
dicarboxylic	Pb^{2+} [c]	1·500	0·990	0·680 ± 0·015	0·453	0·0085	0·340 ± 0·024
	Cu^{2+} [c]	1·500	1·142	0·788 ± 0·010	0·525	0·0089	0·239 ± 0·010

[a] $c_{COOCa_{0.5}} = 3{\cdot}00$ mmol l^{-1}, $I_o = 0{\cdot}0045$ mol l^{-1} (ionic strength unadjusted); [b] $c_{COOK} = 3{\cdot}00$ mmol l^{-1}, $(c_{Pb^{2+}})_o$, $(c_{Cu^{2+}})_o = 1{\cdot}435$ mmol l^{-1}, $I_o = 0{\cdot}010$ (KNO_3); [c] $c_{COOK} = 3{\cdot}00$ mmol l^{-1}, $(c_{Pb^{2+}})_o$, $(c_{Cu^{2+}})_o = 1{\cdot}500$ mmol l^{-1}, $I_o = 0{\cdot}010$ (KNO_3).

TABLE II

Binding of lead and copper(II) cations to D-galacturonic acid (*I*) and its derivatives *IV* and *V*; $c_{COOMo.5} = 3{\cdot}00$ mmol l^{-1}, $I_o = 0{\cdot}010$ mol l^{-1} (KNO_3)

Compound	M^{2+}	$[M^{2+}]_f$ mmol l^{-1}	$a_{M^{2+}} \cdot 10^3$	$\gamma_{M^{2+}}$	I mol l^{-1}	β
I	Pb^{2+} [a]	0·842	0·581 ± 0·012	0·387	0·0080	0·439 ± 0·012
	Cu^{2+} [b]	0·930	0·648 ± 0·011	0·432	0·0083	0·380 ± 0·010
	Pb^{2+} [c]	1·106	0·351 ± 0·002	0·234	0·149	0·263 ± 0·003
	Cu^{2+} [c]	1·144	0·411 ± 0·004	0·274	0·149	0·237 ± 0·007
IV	Pb^{2+}	1·028	0·703 ± 0·005	0·469	0·0085	0·315 ± 0·004
	Cu^{2+}	0·938	0·652 ± 0·012	0·435	0·0083	0·375 ± 0·012
V	Pb^{2+}	1·222	0·826 ± 0·010	0·551	0·0091	0·185 ± 0·010
	Cu^{2+}	1·123	0·774 ± 0·011	0·516	0·0089	0·251 ± 0·010

[a] Ref.[8]; [b] ref.[9]; [c] $I_o = 0{\cdot}150$ mol l^{-1} (KNO_3).

The binding of M^{2+} cations is evaluated in terms of the counter-ion activity $a_{M^{2+}}$ and the degree of association of the M^{2+} ions with the carboxy groups, β. The results are given in Table I and Fig. 1. The degree of association β pertains to the total amount of M^{2+} ions added, irrespective of whether in the system the soluble undissociated complex occurs solely (2,5-furandicarboxylic acid) or in equilibrium with the insoluble salt (galactaric acid).

With galactaric acid, the major fractions of the salts, *viz.* 87·9% calcium salt, 99·4% lead salt and 91·9% copper salt, precipitated from the solutions. The activity coefficients $\gamma_{Ca^{2+}} = 0{\cdot}81$ and $\gamma_{Cu^{2+}} = 0{\cdot}13$ indicate that the small soluble fraction of calcium galactarate is highly dissociated whereas that of the Cu complex is very low dissociated. The activity coefficient of lead ions could not be determined reliably enough because of their very low concentration and activity. 89·5% calcium ions, virtually all (99·9%) lead ions, and nearly all (98·6%) copper ions are bound to the carboxy groups of galactaric acid, predominantly with the formation of insoluble salts in agreement with paper[17].

The salts of 2,5-furandicarboxylic acid are all soluble under the experimental

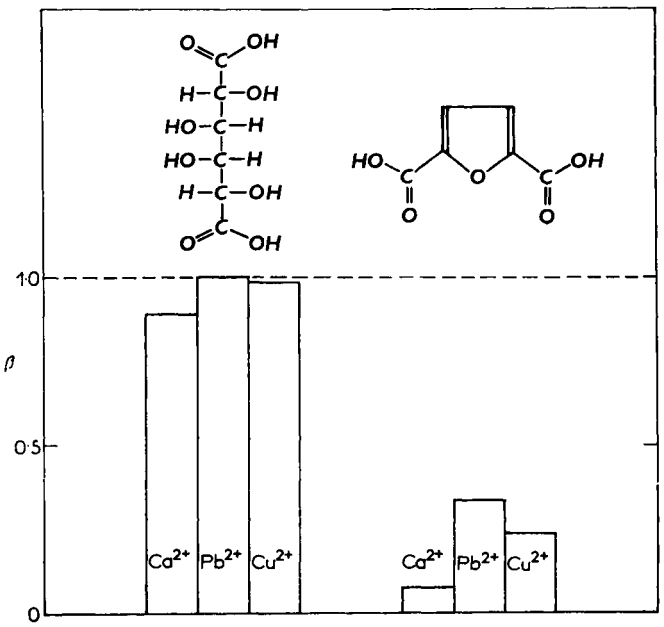

FIG. 1

Binding of calcium, lead, and copper(II) cations to galactaric and 2,5-furandicarboxylic acids. β degree of association

conditions used. Calcium ions are bound to this acid to a low degree only (7·6%), lead and copper are bound to a considerably greater extent (34% Pb, 24% Cu) which, however, is still somewhat less than in the case of the monomeric D-galacturonic acid (44% Pb, 38% Cu; Table II).

The difference in the binding of the cations to galactaric and 2,5-furandicarboxylic acids is consistent with the above-mentioned difference in their molecular arrangement.

Formation of Pb and Cu(II) Complexes of D-Galacturonic Acid

We have shown[11,10] that Ca^{2+}, Sr^{2+}, and Zn^{2+} ions are bound to D-galacturonic acid to a negligible extent ($\beta_{Cu^{2+}} = 0{\cdot}04$, $\beta_{Sr^{2+}} = 0{\cdot}01$, $\beta_{Zn^{2+}} = 0{\cdot}04$). Polymeric D-galacturonan and pectin of various degrees of esterification of the carboxy groups by methanol bind these cations precisely stoichiometrically, *i.e.*, two carboxy groups bind one cation. This stoichiometric binding was also found for Cu^{2+} ions and the monomeric D-galacturonic acid[9], for which the formation of the positively charged $(MA)^+$ complex (where M is a divalent cation and A is the uronic acid anion) has been suggested[28] till now.

All the above measurements were performed at low ionic strengths of the solutions, $I_0 = 0{\cdot}0045 - 0{\cdot}010$ mol l^{-1}. With regard to the use of pectin as a prophylactic against poisoning by toxic cations, the binding of Pb^{2+} and Cu^{2+} cations to D-galacturonic acid was also investigated at an ionic strength of $I = 0{\cdot}15$ mol l^{-1}, corresponding to that of the physiological solution. This binding of cations at different ionic strengths can only be compared in terms of the association degree β because the activity of the counter-ions and, consequently, the activity coefficients vary considerably with varying ionic strength. The β values of the Pb^{2+} and Cu^{2+} ions at $I = 0{\cdot}15$ mol l^{-1} are by 40 and 37·5%, respectively, lower than at $I = 0{\cdot}008$ mol . l^{-1} (Table II); still, at the ionic strength of the physiological solution, 26% of lead ions and 24% of copper ions are bound to the uronic acid.

For the binding of Pb^{2+} and Cu^{2+} cations to polyuronates and pectin, *e.g.* ref.[29], formation of cation complexes with the carboxy groups with the participation of the free electron pairs of the oxygen atoms of the hydroxy groups, oxygen atoms of the pyrane rings of the uronic acid unit, and oxygen atoms of the glycoside bonds is assumed. Aruga[30], who studied the binding of Cu^{2+} ions to D-galacturonic acid by the microcalorimetric method, suggests that in addition to the carboxy group, the oxygen of the pyrane ring takes part in the complexation *via* an electrostatic bond in the outer sphere of the cation. From this aspect we examined the binding of Pb^{2+} and Cu^{2+} ions to some model derivatives of D-galacturonic acid, *viz.* α--methylglycosides of D-galacturonic acid (*IV*) and of 4-deoxy-D-galacturonic acid (*V*), at $I_0 = 0{\cdot}010$ mol l^{-1} (KNO_3). The lead and copper salts of these derivatives are water-soluble.

The results are given in Table II. The following conclusions can be drawn from the association degree values. Potassium D-galacturonate occurs in the solution in an equilibrium of the α and β anomeric forms. The lower $\beta_{Pb^{2+}}$ value for *IV* in comparison with *I* (Fig. 2) suggests that the OH group at $C_{(1)}$ of the β anomer of *I*, lying on the same side of the pyrane ring as the carboxy group, may take part in the *I*–Pb^{2+} complex formation; however, regarding the relatively long distance between this OH group and the carboxy group, the participation of the former in the complexation requires additional experimental evidence. The fact that two molecules of the uronic acid interact with a Pb^{2+} cation also has to be taken into account. A Pb^{2+} ion is thus surrounded by two carboxy groups and two hydroxy groups at $C_{(1)}$ of the β-anomeric form.(In analogy with the stoichiometric binding of Cu^{2+} ions with D-galacturonic acid[9], Pb^{2+} ions can be assumed to be bound stoichiometrically to this acid as well.) In contrast to Pb^{2+} ions, Cu^{2+} ions are bound to *I* and its derivative *IV* to the same extent (Fig. 2), which indicates that the hydroxy group at $C_{(1)}$ does not participate in the complexation of D-galacturonic acid with copper ions.

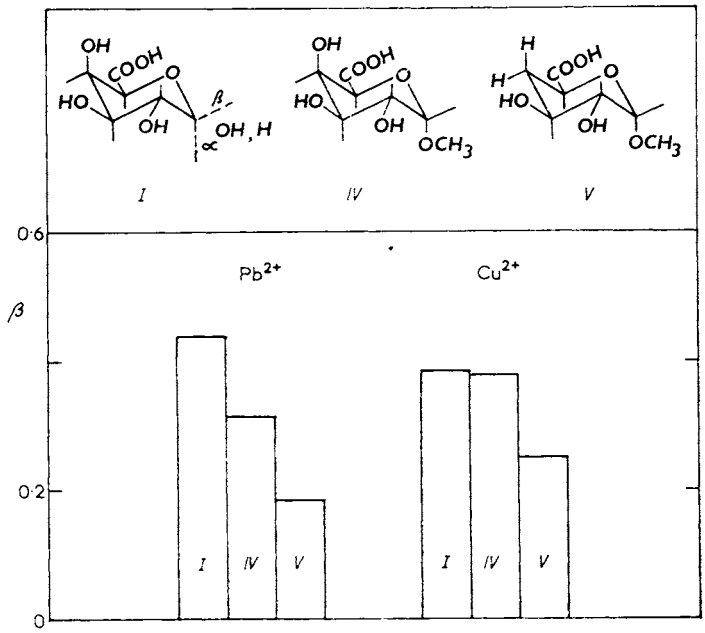

FIG. 2

Binding of lead and copper(II) cations to D-galacturonic acid (*I*) and its derivatives *IV* and *V*. β degree of association

The absence of the hydroxy group at $C_{(4)}$ in the derivative *V* brings about a marked decrease in the β values, as compared with *IV*, both for Pb^{2+} and Cu^{2+} (Fig. 2); this gives evidence that the OH group at $C_{(4)}$ of the unsubstituted acid takes part in the complexation with lead and copper ions.

CONCLUSIONS

The results of this work, together with our previous study[8-11], contribute to the understanding of the binding of some essential and toxic cations (lead in particular) to oligomeric fragments and metabolites of pectin and of the complexation of monomeric D-galacturonic acid with lead and copper(II) cations. The way of how toxic metal cations deposited in the organism are eliminated from the body off the gastrointestinal tract after the peroral administration of pectin is rather intricate, and only further systematic investigation on laboratory animals can reveal the role of pectin degradation and metabolism products in this process; it is hoped that the results of this study may be of assistance in attaining this goal.

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