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Structure of the Galacturonate and Glucuronate Complexes with Copper(II) in Aqueous Solution. A Calorimetric Study

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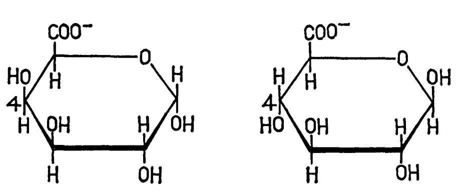
Heat and entropy of reaction of copper(II) with galacturonate and glucuronate anions have been determined by calorimetry. The measurements were performed in an aqueous medium at 25 °C and ionic strength $I=1$ M ($NaNO_3$). The results indicate a bidentate action of the two ligands towards copper. The carboxylate group as well as the oxygen atom of the ring, the latter by means of an outer-sphere electrostatic bond, seem to interact with the cation.

Acid polysaccharides are of importance among the polyelectrolytes of biological interest.[1] Complex formation of these polysaccharides with metal ions gives rise to particular structures and biological functions. Our knowledge of the structures and bonding sites is fragmentary. Data are insufficient for the reactions of not only polysaccharides but also the corresponding acid monosaccharides such as uronic acids. Of the few studies on the metal associations of uronic acids in solution, those of Anthonsen *et al.*[2–4] and Makridou *et al.*[5] may be cited. As regards the bond nature for galacturonate with some metals, the results of the above works do not agree. The NMR data suggest a tridentate behavior towards metal ions of large dimensions, the potentiometric results indicating a monodentate action with only the $-COO^-$ group bound to the metal. The latter conclusion, particularly for copper, appears curious. Thermodynamic studies on the association between this metal and some hydroxy and alkoxy carboxylic acids showed that some groups such as ethereal oxygen or alcoholic –OH group, in particular positions, can form bonds with copper.[6,7]

Taking into consideration the disagreement of these conclusions and the absence of calorimetric studies on metal-uronic acid reactions, it was thought of interest to extend the calorimetric investigations to the reaction of copper(II) with galacturonate and glucuronate (Fig. 1). The results obtained refer to the aqueous medium at 25 °C and $I=1$ M.



I II

Fig. 1. Galacturonate ion (I) and glucuronate ion (II).

Experimental

Materials. Copper(II) nitrate (C. Erba RPE) was used. The concentration of metal was determined by complexometric titration with EDTA (0.1 M solution, C. Erba RPE). Solutions of the ligands were prepared by D(+)-galacturonic acid monohydrate Fluka puriss and D-glucuronic acid Fluka purum. The purity of the acids was checked by potentiometric titration. Inert electrolyte: sodium nitrate (C. Erba RPE).

Equipment. Calorimetric measurements were carried out at 25.00 °C with an LKB 8700-2 Precision Calorimetry System and an LKB 8726-1 100-ml titration vessel equipped with a standard resistor (50Ω) and a thermistor (2000Ω). The accuracy of the instrument was checked by measuring the molar enthalpy of the reaction between tris(hydroxymethyl)methylamine and HCl in aqueous solution. The calorimeter was also equipped with a Radiometer ABU 12b autoburet for the addition of titrant. The calorimetric experiments were carried out in a room whose temperature was kept constant within ±0.3 °C. For the pH measurements a Metrohm Compensator E 388 potentiometer was used at 25 °C.

Procedure. For each ligand several sets of measurements of the reaction and dilution heats were carried out, each set being performed in the following way. 2.5 ± 0.002 ml of 4×10^{-1} M copper nitrate solution was added in succession to 88.00 ml of the uronic acid solution contained in the calorimetric cell, the heat being measured for each addition. The pH of the copper solution was made 3.6 ± 0.1 in order to avoid the metal hydrolysis. The ligands were partly transformed into sodium salt by adding NaOH to $pH=4.55\pm0.02$. The ionic strength of all the solutions was brought to 1 M with sodium nitrate. The corresponding heat of dilution was measured by adding the same amounts of copper nitrate solution to 88.00 ml of 1 M $NaNO_3$, without ligand, at pH=4.55. The following results were obtained. (a) Formation of only the ML^+ complex species; in fact, the presence of only the ML^+ species was shown up to a C_L/C_M ratio of 2.[5] (b) Absence of polynuclear species.[5] (c) A negligible neutralization heat between H^+ and OH^-. The pH value in the cell, after each addition of titrant, was measured potentiometrically by means of titration performed in the same manner as the calorimetric one.

Treatment of the Experimental Data. Owing to the pH difference between metal and ligand solutions, a slight protonation process of the latter took place. The protonation heat was calculated by means of the acid ionization enthalpy of both glycolic acid (0.15 kcal mol^{-1})[8] and acetic acid (0.09 kcal mol^{-1}).[9] It was found to be lower than 0.7% of the measured heat, with no influence on the ΔH° values, and was thus neglected. Data for one series of the measurements: initial concentration of ligand (C_i) and initial pH (pH_i) in the cell, cumulative volume of titrant added (ΣV_T), pH in the cell (pH_f) and concentration of the species (C_{ML^+}, C_{L^-}, C_{HL}) after each addition of titrant, and cumulative heat corrected for the dilution (ΣQ_c) are given as an example in Table 1.

The molar enthalpy of association was determined from the heat (ΣQ_c) experimentally obtained and the complex species concentrations, the latter being obtained from the stability constants.[5] The calculation of ΔH° was carried out by two methods:[7,10] (a) the numerical method of minimization of the error square sum, and (b) the graphical

TABLE 1. DATA FOR THE MIXING OF Cu(II) WITH GALACTURONATE AND GLUCURONATE SOLUTIONS

Ligand	$\frac{C_i}{10^{-2}\,\mathrm{M}}$	$\mathrm{pH_i}$	$\frac{\Sigma V_T}{\mathrm{ml}}$	$\mathrm{pH_f}$	$\frac{C_{ML^+}}{10^{-3}\,\mathrm{M}}$	$\frac{C_{L^-}}{10^{-2}\,\mathrm{M}}$	$\frac{C_{HL}}{10^{-4}\,\mathrm{M}}$	$\frac{-\Sigma Q_c}{\mathrm{cal}}$
Galacturonate ion	2.50	4.57	2.505	4.33	5.81	1.73	12.1	0.318
			5.010	4.20	9.69	1.28	12.1	0.518
Glucuronate ion	2.51	4.56	2.505	4.43	4.11	1.96	6.50	0.309
			5.010	4.31	7.00	1.60	6.99	0.533

TABLE 2. THERMODYNAMICS FOR THE REACTION: $Cu^{2+} + L^- \longrightarrow CuL^+$, IN AQUEOUS SOLUTION AT 25 °C AND $I = 1$ M

L^-	$\log K$	$\frac{-\Delta G^{\circ}\ ^{a)}}{\mathrm{kcal\ mol^{-1}}}$	$\frac{\Delta H^{\circ}}{\mathrm{kcal\ mol^{-1}}}$	$\frac{\Delta S^{\circ}}{\mathrm{gibbs\ mol^{-1}}}$	$(\log K)/\mathrm{p}K_a$	Ref.
Galacturonate	$1.81\pm0.02^{b)}$	2.46 ± 0.03	0.60 ± 0.04	10.3 ± 0.2	0.57	This work
Glucuronate	1.48 ± 0.03	2.01 ± 0.04	0.82 ± 0.01	9.5 ± 0.1	0.50	This work
Methoxyacetate	1.83	2.49	0.74 ± 0.04	10.8	0.56	6)
Ethoxyacetate	1.80 ± 0.02	2.45 ± 0.03	0.50 ± 0.02	9.9 ± 0.1	0.51	7)
Pyruvate	1.59	2.17	-0.05 ± 0.01	7.1	0.50	6)
Glycolate	2.17	2.96	-0.35 ± 0.01	8.7	0.62	6)
Acetate	1.33	1.81	1.24 ± 0.02	10.2	0.30	6)

a) The results are expressed by means of the thermochemical calorie, equal to 4.1840 absolute J. b) The uncertainty in each case is given as the estimated standard deviation.

method derived from the equations of Leden and Fronaeus for the overall formation constants. The results agree. The entropy was obtained by means of: $\Delta S^{\circ} = 10^3(\Delta H^{\circ} - \Delta G^{\circ})/T$.

Results and Discussion

The molar thermodynamic quantities for the reactions of copper(II) with galacturonate and glucuronate are given in Table 2. For the sake of comparison, the values obtained for a series of monocarboxylic ligands[6,7] (under the same conditions of T and I) are also given.

The values in Table 2 for the uronate anions show that only the entropy factor is favorable for the association but not ΔH°. The positive values of these parameters can be caused by two factors: (a) partial solvent destruction accompanying the charge association, and (b) the displacement of at least one water molecule by carboxylate from the metal hydration sphere.[11] Nevertheless, the crystal fields produced by oxygen coordinating ligands are quite similar to those of the water molecules.[11] As regards the entropy variation, the displacement of one water molecule is counterbalanced by the metal-ligand association. Consequently it seems that both ΔH° and ΔS° are mainly influenced by factor (a).

One of the following arrangements might take place as regards the metal-ion interaction: (a) monodentate behavior of the uronate ions, with carboxylate group as the only bonding site, the structure being proposed on the grounds of the formation constants values;[5] (b) bidentate behavior, with $-COO^-$ and $-OH$ on C-4 as bonding sites; (c) bidentate action as in (b), but with $-COO^-$ and the ethereal oxygen of the ring taking part in the binding; (d) a tridentate action, by means of $-COO^-$, $-OH$ on C-4 and the oxygen of the ring, the structure being suggested for the association of galacturonate with Ca(II) and Eu(III) ions.[2]

Considering the fact that the log K values for uronate ions are greater than those for acetate (in spite of the latter's greater basicity towards proton), assumption (a) appears to be questionable. For the sake of illustration the values of $(\log K)/\mathrm{p}K_a$ are given for each ligand (Table 2). The $(\log K)/\mathrm{p}K_a$ ratio allows a comparison, for each ligand, between the stability of its association with copper and with proton. If a ligand acts as a monodentate towards copper (*i.e.*, the bond only through carboxylate is formed), the other groups in the ligand molecule have the same solvent ordering ability both in the case of copper and of proton. If a ligand acts as a bidentate towards the metal, another hydrophilic group besides carboxylate is neutralized by the metal charges. Consequently, the solvent ordering ability of this group is different before and after the reaction. In this case its external contribution to ΔG° of association with copper differs from that with proton. In the same way, the ratio $(\log K)/\mathrm{p}K_a$ should differ as compared with the former case. Therefore the value of this ratio should be determined by a mono- or bidentate behavior of the ligand towards copper (last column, Table 2). For acetate the value is 0.30, while the other ligands (for which a bidentate action was demonstrated) give values in the range 0.50—0.62. The values of $(\log K)/\mathrm{p}K_a$ for uronate ions (0.57 and 0.50) tend to discard the hypothesis of a monodentate action of galacturonate and glucuronate towards copper.

As regards hypothesis (b), it must be considered that in uronic acids the respective positions of $-OH$ on C-4 and $-COO^-$ are the same as in 3-hydroxypropionic acid. The monodentate action of this ligand towards Cu(II) and other bivalent cations, and the consequent non-participation of –OH in the β-position in the binding of metal were demonstrated.[12,13] Considering also that –OH group, if taking part in complex formation, leads to exothermic values of ΔH° (the values for glycolate, Table 2), the suggestion in (b) may be unlikely.

The structure in (d) does not seem very reliable, both for the preceding considerations on –OH in β-position and for the following reasons. In a tridentate structure there is a strong difference in stability between the galacturonate complex (more stable) and that of glucuronate. This might be due to the different positions, in the molecule of the two ligands, of the hydroxyl group on C-4, which is axial and equatorial, respectively.[5] On the contrary, similar values of $\log K$, ΔH°, and ΔS° (Table 2) lead to the conclusion that both uronate ligands have the same behavior. Moreover, in the case of galacturonate, it was found[2] that a structure with three bonding sites fits the cations of particular dimensions, as Eu(III) and Ca(II) (crystalline radii: 0.95 and 0.99 Å, respectively), while Cu(II) is markedly smaller (0.72 Å, Ref. 14).

The structure given in (c) seems to be probable, considering the fact that the respective positions of ethereal oxygen and carboxylate group are the same as in methoxy- and ethoxyacetate, and that the participation of ethereal oxygen in the chelation of Cu(II) was confirmed for these two ligands.[6,7] In this case the metal-ethereal oxygen bond appeared to be of electrostatic character, and limited to the outer sphere of the cation. Similar values of $\log K$, ΔH°, and ΔS° between the two uronic and the two alkoxyacetic ligands (in particular, the values for galacturonate are between those for the two alkoxyacetates), support the last hypothesis.

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