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Complexing of carbohydrates with copper ions: a reappraisal*†

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ABSTRACT

Contrary to statements in the literature, Cu^{2+} ions form only weak complexes with polyols. At pH > 5, however, strong complex formation is observed which is attributed to $[Cu_2(OH)_2]^{2+}$ or similar binuclear ions. The formation of tetradentate complexes is postulated with compounds having four hydroxyl groups in suitable steric relationship, especially alditols.

INTRODUCTION

The pioneering work of Reeves[2] on formation of complexes of sugars with cuprammonium solution was the first attempt to establish correlations between complexing ability and the configurations and conformations of the sugars. Distinct changes were found in physical properties, due to complex formation, whenever the sugar had two vicinal *gauche* hydroxyl groups or two *syn*-axial hydroxyl groups. The strongest complex was formed by 1,6-anhydro-β-D-mannopyranose[3], the only one of the compounds studied by Reeves which had an *ax,eq,ax* sequence of three oxygen atoms, an arrangement now recognized as a good complexing site[4].

Reeves found the stability constant of this complex to be 250 M^{-1}, a value about two orders of magnitude greather than those found later for other cations in neutral solution[4]. However, aqueous cuprammonium contains a large excess of ammonia and there can be little doubt that it is not the sugars, but anions derived therefrom, which form complexes with the copper tetramine ion.

The complexing ability of the cuprammonium ion in neutral aqueous solution can be tested by the technique introduced into carbohydrate chemistry by Weigel and his co-workers[5], namely t.l.c. on plates coated with a cation-exchange resin. When such a plate is immersed in aqueous copper sulfate and subsequently dipped into conc. aqueous ammonia, it turns bright blue and its chromatographic properties are altered. Undoubtedly, the resin has been converted into the cuprammonium form. Polyols with suitable complexing sites are adsorbed strongly and the R_F values are mostly a little lower than those obtained on plates in the Ca^{2+} form[5,6] (for example, xylitol, 0.45 – 0.50;

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mannitol, 0.56; allitol, 0.64; 1,6-anhydro-β-D-glucopyranose, 0.67). The exceptionally strong complexing with cuprammonium ions found by Reeves is due to the alkalinity of the solution. There are other reports of the formation of complexes of copper ions with carbohydrates in strongly alkaline solution and, undoubtedly, the complexing entity is the cuprate ion[7].

Also, there are numerous reports on complex formation in neutral solution. Thus, Bourne and his co-workers[8] reported the electrophoretic mobilities in aqueous copper acetate of numerous polyhydroxy compounds and their separations on the copper form of cation-exchange resins. Weigel and his co-workers[5] found that t.l.c. plates immersed in aqueous copper acetate gave lower R_F values than those made with calcium acetate, and Haas[9] found that copper ions formed stronger complexes than did calcium ions. On the other hand, Angyal and Mills[6] listed Cu^{2+} amongst the cations which complex weakly, and Vesala and co-workers[10] concluded from measurements of the enthalpy of complex formation that Cu^{2+} does not complex significantly with several methyl pentofuranosides. Therefore, the data in the literature are conflicting, and a reappraisal is now reported.

DISCUSSION

Mills[11] carried out electrophoresis in aqueous copper acetate and copper sulfate, and good mobilities were found only in the former. When thin-layer ion-exchange plates were immersed in aqueous copper sulfate and copper chloride, they did not complex with polyols and the R_F values were >0.8 (except for *cis*-inositol, R_F 0.21). Similarly, a cation-exchange column, converted into its copper form with copper sulfate, failed to separate the components of mixtures of sugars. It appears, therefore that the ions in an aqueous solution of copper acetate form complexes with polyols but those in aqueous copper sulfate do not. Bourne and his co-workers[8] and Weigel and his co-workers[5] used aqueous copper acetate.

There can be no doubt that aqueous copper sulfate and copper chloride contains Cu^{2+} ions, since these salts are almost completely dissociated in water. Thus, it appears that Cu^{2+} ions do not form strong complexes with polyols, as is to be expected since the ionic radius of Cu^{2+} is only 72 pm, which is about the same as that of Mg^{2+} which forms only weak complexes.

Copper acetate, on the other hand, is only partially dissociated according to the scheme

$$Cu(OAc)_2 \rightleftharpoons CuOAc^+ + AcO^- \rightleftharpoons Cu^{2+} + 2AcO^-.$$

The equilibrium constants are known[12] and, in an aqueous 5% solution, ~62% of the copper acetate is undissociated, ~36% is present as the monocation, and only ~2% as Cu^{2+}. Therefore, it appeared possible that $CuOAc^+$ is the cation that forms strong complexes with polyols. In order to test this hypothesis, an ion-exchange column was treated with aqueous copper acetate and eluted with dilute hydrochloric acid, and the eluate was analysed for copper and for acetic acid. It was found that the column had contained 0.10 mequiv. of copper per mL of resin but only 0.003 mequiv. of acetate per mL. Therefore, the $CuOAc^+$ ion is only a minor component amongst the copper-containing cations on the column.

At pH >4.0, Cu^{2+} ions are increasingly coordinated to hydroxyl ions, forming a variety of cations, $[Cu_2(OH)_2]^{2+}$ being preponderant. This ion appears to be $[Cu_2(OH)_2(H_2O)_4]^{2+}$ but the water of hydration will not be indicated in the ensuing discussion. The formation constant of this ion is known[13], and calculation shows that, in aqueous 5% copper acetate, the ratio of Cu^{2+} to $[Cu_2(OH)_2]^{2+}$ is 500:1 at pH 5, and 7:1 at pH 6; at pH 4, the proportion of the binuclear ion is negligible. The pH of aqueous 5% copper sulfate is 3.6 and that of aqueous 5% copper acetate is 5.0. Hence, the former contains negligible amounts of the binuclear ion, and the latter a substantial proportion. Therefore, the significant difference between the two solutions is not the nature of the anion but their pH. Haas[9] measured the complexing of copper ions at pH 6.1.

The effect of varying the pH can be clearly seen in the electrophoretic mobilities found by Mills[11] (Table I). Addition of acetic acid to aqueous copper acetate reduces the mobilities which indicates weakened complex formation. Addition of acetic acid lowers the pH but does not otherwise affect the formation of complexes.

If the complexing ion is indeed $[Cu_2(OH)_2]^{2+}$, increasing the pH would promote the formation of complexes. Adding even a small amount of sodium hydroxide to aqueous copper sulfate causes immediate precipitation, but up to 0.4 equiv. can be added to aqueous copper acetate without causing more than cloudiness. This solution then has a pH of 5.6 and can be used for converting the t.l.c. plate or the ion-exchange column into the copper form, but it has to be used immediately because, after a few hours, crystallization occurs and a day later the solution sets almost solid with fine needles of, presumably[15], $Cu(OAc)_2 \cdot Cu(OH)_2 \cdot 5H_2O$.

Treatment of the t.l.c. plate in this way results in lower R_F values than that with copper acetate alone (Table II). The color of the plates prepared with copper sulfate is pale grey, with copper acetate, pale green, and with copper acetate plus sodium hydroxide, greenish blue.

TABLE I

Electrophoretic mobilities (M_i) of some alditols in various electrolytes[a]

Alditol	*0.25M $Cu(OAc)_2$*	*0.25M $Cu(OAc)_2$ + 0.2M AcOH*	*0.25M $Cu(OAc)_2$ + 0.4M AcOH*	*0.2M$Cu(OAc)_2$ + 0.2M AcOH*[b]
Xylitol	0.90	0.60	0.40	0.18
D-Glucitol	0.74	0.60	0.13	0.20
D-Iditol	0.80	0.74	0.25	0.24
Galactitol	0.76	0.22	0.05	0.17
Allitol	0.34	0	—	0.09

[a] Ref. 11: paper electrophoresis for 2 h with a current of ~18 mA. [b] Ref. 14.

TABLE II

R_f values of some polyols on a t.l.c. plate prepared with copper acetate (*A*) and with copper acetate + 0.4 equiv. of sodium hydroxide (*B*)

Compound	A	B
D-Glucose	0.90	0.82–0.85
D-Allose	0.48–0.56	0.44–0.50
D-Altrose	0.78	0.34–0.51
D-Ribose	0.45–0.57	0.11–0.22
D-Mannitol	0.36–0.58	0.17–0.50
myo-Inositol	0.60	0.17–0.48

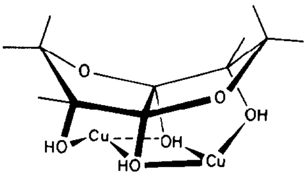
It was found fortuitously that Cu^{2+} ions can be separated from the binuclear ions, once they are adsorbed on the ion-exchange resin. In order to determine how much copper a column contained, it was eluted with aqueous sodium acetate. The appearance of blue color in the eluate indicated that Cu^{2+} ions were replaced by Na^+ ions, but, when the eluate was no longer blue, the column still had a dark green color and was still capable of separating the constituents of mixtures of sugars. The complex-forming ion, presumably $[Cu_2(OH)_2]^{2+}$, therefore, is not eluted from the ion-exchange resin by Na^+ ions, but it can be removed by elution with strong acids. This ion is adsorbed more strongly than monoatomic cations, probably because it is co-ordinated to two anionic centres on the surface of the resin. Thus, although the binuclear ion occurs in a low proportion in aqueous copper acetate, it is adsorbed preferentially by the resin and, as it is depleted in solution, more is formed to restore the equilibrium.

It appeared that the proportion of the complex-forming cation on the resin could be increased further by eluting the resin with aqueous 5% sodium acetate, and then re-treating with aqueous copper acetate plus sodium hydroxide. Repeating this process three times gave a t.l.c. plate which was bright blue, and resulted in a further decrease of R_F values. Selective removal, by sodium acetate, of Cu^{2+} ions from a column thus prepared allowed the determination of the residual proportion of copper on the column which, presumably, is complex-forming. For a column treated with copper acetate, the value was 19%; for one treated with copper acetate plus sodium hydroxide, 33%; and for one treated four times in this way, 68%.

The R_F values on copper t.l.c. plates do not always parallel those observed on Ca^{2+} plates. In particular, some alditols complex much more strongly on the former. This result seems to indicate that the structures of the two complexes are different.

The structure of the $[Cu_2(OH)_2]^{2+}$ ion does not seem to be known. It has been suggested[16], without much evidence, that it is linear. However, numerous crystal structures are known[17] in which this cation appears as two copper atoms bridged by two oxygen atoms but, in each structure, one of the atoms coordinated to copper is nitrogen. The two copper and the two oxygen atoms form a four-membered ring, with Cu–O distances of 190–195 pm and bond angles of 75–80° on the copper and 98–105° on the oxygen atoms as, for example, in the crystal structure of the complex between copper ions and diethylaminoethanol. It appears reasonable that the copper and oxygen atoms assume the same configuration in complexes involving only oxygen atoms.

Furanoses with three contiguous *cis* hydroxyl groups complex strongly with cations[4]. However, D-*threo*-3,4-hexodiulose, with four *cis* hydroxyl groups in its α form, complexes weakly[18] (R_F 0.70 with Ca^{2+}) since the rigidity of the fused ring system does not allow appropriate approach of the hydroxyl groups. On a t.l.c. plate prepared with copper acetate, strong complex formation was observed (R_F 0). Assuming the above-described geometry for the Cu_2O_2 entity, the structure **1** can be built in which the binuclear cation complexes with all four hydroxyl groups without any strain.



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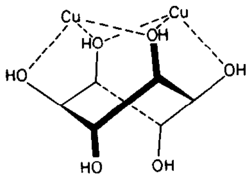

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muco-Inositol does not have an *ax,eq,ax* sequence of hydroxyl groups and therefore forms only weak complexes with cations (R_F 0.72 with Ca^{2+}). On the copper t.l.c. plate, strong complexing is observed (R_F <0.07). The binuclear copper cation can readily be fitted to the four *cis* hydroxyl groups in the flexible form (**2**) of *muco*-inositol. 1-*O*-Methyl-*muco*-inositol, with one of the *cis* hydroxyl groups blocked, forms a much weaker complex (R_F 0.69).

Further evidence about the formation of copper complexes can be gained from the behaviour of alditols. The values listed by Weigel and his co-workers[5] show that several 1-deoxyalditols have R_F values higher than those of the parent alditols. The R_F values of other 1-deoxyalditols (see Table III) have been measured and this relationship was found to be true, generally, for copper t.l.c. plates. It is not true for Ca^{2+} and other monoatomic cations. Removal of the primary hydroxyl group does not affect the extent of complex formation (Table III). Therefore, the primary hydroxyl group is not

TABLE III

R_F values of some alditols on copper and calcium forms of t.l.c. plates

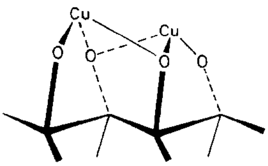
Compound	*Cu*[a]	*Ca*
Xylitol	0.03–0.08	0.68
–, 1-deoxy	0.30–0.40	0.68
L-Arabinitol	0.34–0.42	0.73
–, 1-deoxy	0.48–0.62	0.73
D-Glucitol	0.05–0.10	0.69
–, 1-deoxy	0.19	0.69
–, 6-deoxy	0.25–0.30	0.69

[a] Prepared with copper acetate.

involved in complex formation. The behaviour of the copper cations is reminiscent of the complexing of polyols with molybdate and tungstate cations, where 1-deoxyalditols also form complexes weaker than those of the parent alditols[19,20]. These complexes are known to be tetradentate[21] and to contain two metal atoms bridged by oxygen atoms[22].

The behaviour of xylitol is noteworthy. Formation of a tetradentate complex must involve one of the primary hydroxyl groups. 1-Deoxyxylitol forms a complex much weaker than that of xylitol (Table III). This behaviour strongly suggests the formation of a tetradentate complex with a copper-containing cation. For hexitols, where tetradentate complexes involving only secondary hydroxyl groups are possible, the effect of removing a primary hydroxyl group is less pronounced but is still strong.

If the complexing cation has a four-membered ring of copper and oxygen atoms, as detailed above, only iditol amongst the hexitols can form a complex (**3**) at O-2/5 in the (most stable) planar zigzag conformation. The two oxygen atoms bridging the copper atoms are vicinal on the alditol chain, as in the postulated structure for the complex **1** with D-*threo*-3,4-hexodiulose. This option is possible only if O-3 and O-4 are *threo*. If they are *erythro*, a different arrangement is possible in which the bridging oxygen atoms are not vicinal, as shown in formula **4** for the complex with galactitol. This is the arrangement postulated for the complex **2** of *muco*-inositol. It appears from the R_F values that the *threo* complexes are more favourable. On the other hand, molybdate complexes appear to favor the structure in which the bridging oxygen atoms are not vicinal[22], probably because the Mo–O bond is longer than the Cu–O bond.



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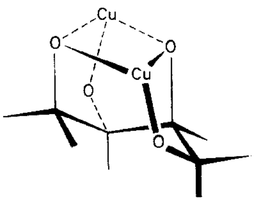

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The complexes formed with galactitol and glucitol have one *gauche* arrangement in the chain, those with mannitol and altritol have two, and that with allitol has a most unfavorable conformation. The extent of complex formation was found to vary in the order predicted. However, the formation of more than one complex is possible. As the number of *gauche* interactions increases, complexes involving the primary hydroxyl groups will become significant since they do not cause unfavorable interactions. Many alditols form more than one complex with molybdate ions, some of which involve primary hydroxyl groups.

In these assumed structures, each copper atom is coordinated to three oxygen atoms, as found in molybdate and tungstate complexes. A rough criterion for the formation of tetrahedral complexes is a ratio of >2 for R_F(Ca) to R_F(Cu), for example, 4.4 for D-talose, 5.1 for galactitol, and 5.1 for xylitol. For compounds that cannot form tetradentate complexes, this ratio is usually 1.1–1.3, *i.e.*, the extent of complex formation with calcium and with copper ions is similar.

In order to confirm the suggested tetradentate structure, n.m.r. line-broadening experiments were carried out. Glucitol forms complexes[23] with monoatomic cations at O-2,3,4. Indeed, addition of a small amount of gadolinium chloride, a line-broadening agent[24], caused broadening of the ^{13}C signals of C-2,3,4; the signal of C-1 broadened only slightly more than that of C-6. On the other hand, addition of a small amount of copper acetate broadened the signals of C-1,2,3,4 and the signal of C-1 broadened more than that of C-2.

Although the evidence presented herein is circumstantial, there seems to be strong support for the suggestion that ions in a copper solution at pH >5 form tetradentate complexes with suitable polyols. An X-ray crystal structure determination of a complex would provide decisive evidence but, although it is easy to prepare complexes of alditols with copper acetate, all were amorphous.

The R_F values on t.l.c. plates treated with copper acetate are mostly larger than those reported by Weigel and his co-workers[5]. Despite several discussions with Dr. Weigel, the reason for this difference has not been established. Possibly the commercial plates available in the 1970s and 1980s were different. The spots shown by the compounds with small R_F values were not sharp and there was considerable streaking, particularly with alditols, and the reproducibility of the results was unsatisfactory. Some alditols show up as blue spots or streaks even before being sprayed with permanganate. Evidently, the complex has a darker color than the uncomplexed cation. The reason for the streaking is not clear. It may well be that the formation of tetradentate complexes, which requires a certain orientation of the cation, is slower than that with spherical cations. T.l.c. at 50–60° improved the appearance of the spots but did not eliminate the streaking completely. Streaking may also be caused by the presence of more than one complexing cation since, in addition to $[Cu_2(OH)_2]^{2+}$, small amounts of other combinations of Cu^{2+} and HO^- are present[16] in copper solutions at pH >5.

EXPERIMENTAL

Polygram Ionex-25 SA-Na sheets (Macherey–Nagel) were immersed in deionized water, then in aqueous 5% copper acetate for 1 h, and then washed several times with deionized water. For plates prepared at higher pH, the aqueous copper acetate was mixed with 10% of its volume of M NaOH. After developing the chromatograms with deionized water, the plates were dried at room temperature, and the compounds were detected by spraying with a saturated solution of potassium permanganate in acetone. Sometimes, strongly complexing compounds failed to show up after spraying, and the plate was then sprayed with 1:10 conc. hydrochloric acid and acetone in order to decompose the complex, dried, and again sprayed with permanganate. The plate rapidly turns brown, and white spots of the polyols appear in 10–24 h.

^{13}C-N.m.r. spectra of D-glucitol were recorded with a Bruker AM-500 spectrometer. Aliquots of aqueous 1% gadolinium chloride were added to 0.5M D-glucitol in D_2O. The line-widths at half-heights of the ^{13}C signals[25] were 2.2–2.7 Hz. Gadolinium broadened the signals of C-3 the most. Those of C-4 and C-5 fused when broadened and their width could not be measured, but it appears that the signal of C-5 broadened little (the height was not reduced). Addition of 0.0010 and 0.0020 mole % of gadolinium chloride caused the following broadened line-widths: C-2, 8.3 and 11.7 Hz; C-3, 10.1 and 17.2 Hz; C-6, 5.0 and 5.5 Hz; and C-1, 5.0 and 6.7 Hz.

Addition of 0.01, 0.11, and 0.22 mole % of copper acetate caused the following broadened line-widths: C-2, 4.4, 10.0, and 13.9 Hz; C-3, 5.5, 11.0, and 19.4 Hz; C-6, 3.9, 7.8, and 11.7 Hz; and C-1, 4.4, 11.1, and 15.6 Hz.

REFERENCES

1 S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 38 (1985) 1279–1285.
2 R. E. Reeves, *Adv. Carbohydr. Chem.*, 6 (1951) 107–134.
3 R. E. Reeves, *J. Am. Chem. Soc.*, 73 (1951) 957–959.
4 S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 1–43.
5 J. Briggs, P. Finch, M. C. Matulewicz, and H. Weigel, *Carbohydr. Res.*, 97 (1981) 181–188.
6 S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 32 (1979) 1993–2001.
7 P. Pfeiffer, H. Simons, and E. Schmitz, *Z. Anorg. Allg. Chem.*, 256 (1948) 318–342.
8 E. J. Bourne, F. Searle, and H. Weigel, *Carbohydr. Res.*, 16 (1971) 185–187.
9 J. W. Haas, Jr., *Mar. Chem.*, 19 (1986) 299–304.
10 A. Vesala, H. Lönnberg, K. Käppi, and J. Arpalahti, *Carbohydr. Res.*, 102 (1982) 312–315.
11 J. A. Mills, unpublished data.
12 *Stability Constants, Suppl. 1*, Chem. Soc. Special Publications 25, London, 1951, p. 251.
13 R. K. Steinhaus and C. L. Barsuhn, *Inorg. Chem.*, 13 (1974) 2922–2929.
14 S. J. Angyal, D. Greeves, and L. Littlemore, *Aust. J. Chem.*, 27 (1974) 1447–1456.
15 J. Gauthier, *C. R. Acad. Sci., Ser. A*, 242 (1956) 644–647.
16 H. Ohtaki and T. Kawai, *Bull. Chem. Soc. Jpn.*, 45 (1972) 1735–1738.
17 H. Melník, *Coord. Chem. Rev.*, 42 (1982) 259–293.
18 S. J. Angyal, D. C. Craig, and J. Kuszmann, *Carbohydr. Res.*, 194 (1989) 21–29.
19 H. J. F. Angus, E. J. Bourne, and H. Weigel, *J. Chem. Soc.*, (1965) 21–26.
20 J. Briggs, I. R. Chambers, P. Finch, I. R. Slaiding, and H. Weigel, *Carbohydr. Res.*, 78 (1980) 365–367.
21 M. Matulová, V. Bílik, and J. Alföldi, *Chem. Papers*, 43 (1989) 403–414.
22 J. E. Godfrey and J. M. Waters, *Cryst. Struct. Commun.*, 4 (1975) 5–8, and unpublished results.
23 A. P. G. Kieboom, A. Sinnema, J. M. van der Toorn, and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 96 (1977) 35–37.
24 K. Dill and R. D. Carter, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 125–166.
25 S. J. Angyal and R. Le Fur, *Carbohydr. Res.*, 84 (1980) 201–209.