[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, LOUISIANA STATE UNIVERSITY SCHOOL OF MEDICINE]

Cupranmonium-Glycoside Complexes. VIII. The Copper to Diol Combining Ratio[1]

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Continuous variation studies with cuprammonium and solutions of eight cyclic diols, glycerol, and eleven carbohydrate pyranosides indicate that the copper to diol combining ratio is one to one. Di-D-fructopyranose 1,2'-2,1'-dianhydride, sucrose, and methyl α-D-galactopyranoside each contain two reactive diol sites and, depending upon the reaction conditions, the last two substances may react at one or both sites. Potentiometric titrations show that at least one proton is liberated upon cuprammonium-diol chelation and it is concluded that one of the reactive species is the singly charged diolate anion.

In preceeding papers of this series[2] evidence has been gathered regarding the capability of diol groupings, notably those occurring in glycosides, to form bidentate complexes (chelates) with copper in cuprammonium hydroxide solutions. Hypotheses have been developed which correlated some of the observed chelate properties with the relative orientation of the reactive diol groups and, indirectly, with the conformations existing in the glycoside molecules. Further progress in this direction is handicapped by the paucity of existing information concerning the reactive copper species, the reactive diol species, and the composition and structure of the diol chelates.

In order to explore by chelation studies the differences in the shapes of molecules such as the glycosides it would be advantageous to be able to determine and compare their equilibrium constants for the cuprammonium-diol chelation reaction. This would require, among other things, reliable information on the copper-diol combining ratios. McDonald has recently considered this problem and has reviewed its historical aspects, pointing out that while the combining ratio has generally been assumed to be one to one there is meagre evidence to support this contention.[3] McDonald's experimental approach employed continuous variation studies, the results of which led her to suggest that two atoms of copper combine with each reactive diol grouping, a conclusion which does not find support in the present work.

Potentiometric titrations. To explore the diol chelation reaction potentiometric titrations were carried out in which sodium hydroxide was titrated against cuprammonium solutions with and without added 1,4-anhydroerythritol. These results showed that one proton was liberated in the diol-containing system at a *p*H approximately two units lower than would be expected from the acid dissociation constant of the diol. This suggests that the reactive species is the singly charged diolate anion, a finding of significance to the continuous variation experiments for it implies that such studies should be carried out at a constant *p*H. Otherwise, the diol to diolate-anion ratio would vary during the course of the experiments and obscure or invalidate the observations.

Continuous variation studies with cyclic diols. Preliminary to attacking the problem of the cuprammonium-to-glycoside combining ratios studies were made on a number of cyclic diols, choosing substances known to react strongly or moderately actively with cuprammonium. All but the most reactive diols require highly alkaline conditions in order to secure extensive interaction, conditions under which *p*H measurement is both difficult and uncertain. In lieu of accurate *p*H control the expedient was adopted of adjusting the strong alkali concentrations of both of the titrating solutions to equivalence. The copper(II) and ammonia of the cuprammonium solution were adjusted to equal the diol and ammonia concentrations, respectively, of the diol solution; and sodium hydroxide was added to the diol solution to approximately equal the strong alkali, cupric hydroxide plus free sodium hydroxide (if any), in the cuprammonium solution. Omission of the added alkali from the diol solutions resulted in the reaction being most favored when the cuprammonium content of the mixed solutions was greatest, and this produced asymmetric continuous-variation curves which were not interpretable in terms of copper to diol combining ratio.

Illustrated in Fig. 1 (curve A) is a typical continuous variation experiment employing *endo, endo*-2,3-dihydroxybicyclo[2.2.1]heptane as the diol-containing substance. These results clearly demonstrate the one-to-one combining ratio between copper and the diol. Other diols yielding results indicative of this ratio were the following (concn. Cu plus diol): *cis*-cyclohexane-1,2-diol($0.1M$); *cis*-cyclopentane-1,2-diol($0.02M$); 1,1-dimethyl-*cis*-cyclopentane-2,3-diol($0.02M$); *exo-exo*-2,3-dihydroxybicyclo[2.2.1]heptane ($0.02M$); *exo-exo*-2,3-dihydroxy - 1,7,7 - trimethylbicyclo[2.2.1]heptane ($0.02M$); 1,4-anhydroerythritol (0.02, $0.10M$); and *exo*-2-*syn*-7-dihydroxybicyclo[2.2.1]heptane

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[1] Supported in part by a grant from the Corn Industries Research Foundation.

[2] Part VII, R. E. Reeves, *J. Am. Chem. Soc.*, **76**, 4595 (1954).

[3] E. J. McDonald, *J. Org. Chem.*, **25**, 111 (1960).

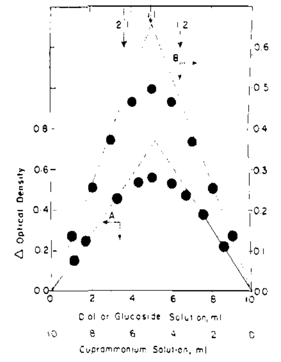

Fig. 1. Continuous-variation experiments with optical density as the variable property.

A. *endo,endo*-2,3-Dihydroxybicyclo[2.2.1]heptane, $0.02M$, in $3M$ ammonia containing $0.10M$ sodium hydroxide *vs.* $0.02M$ cupric nitrate in $3M$ ammonia containing $0.14M$ sodium hydroxide; 360 mμ

B. Methyl α-D-glucopyranoside, $0.10M$, in $6M$ ammonia containing $0.20M$ sodium hydroxide *vs.* $0.10M$ cupric hydroxide in $6M$ ammonia; 370 mμ

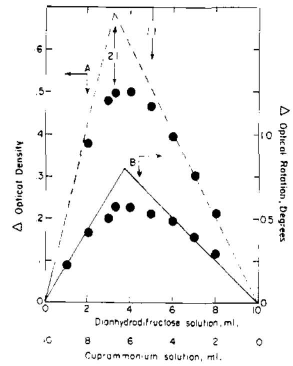

Fig. 2. Continuous-variation experiments employing $0.04M$ di-D-fructopyranose 1,2′-2,1′-dianhydride in $6M$ ammonia containing $0.08M$ sodium hydroxide *vs.* $0.04M$ cupric nitrate in $6M$ ammonia containing $0.08M$ sodium hydroxide.

A. Optical density at 360 mμ as the variable property

B. Optical rotation at 436 mμ as the variable property

($0.02M$). The last substance is of particular interest in that the two hydroxyl groups are not located on contiguous carbon atoms.

Studies with glycosides and related substances. Continuous variation experiments with methyl α-D-glucopyranoside, illustrated in curve B of Fig. 1, yielded a symmetrical curve indicative of a 1:1 copper to glucoside ratio. Other carbohydrate derivatives which were investigated and found to react with copper at the 1:1 ratio were the following (concn. Cu plus diol-containing substance): glycerol ($0.1M$); methyl 4,6-*O*-benzylidene-α-D-glucopyranoside($0.02M$); 1,6-anhydro-β-D-mannopyranose ($0.02M$); methyl 2,6-anhydro-α-D-altropyranoside ($0.02M$); phenyl2, 3-di-*O*-methyl-β-D-galactopyranoside($0.1M$); methyl α-D-xylopyranoside($0.08M$); methyl β-L-arabinopyranoside($0.1M$); and 1,6-anhydro-β-D-glucopyranose($0.02M$). Glycerol was included in this series because it, like the simple glycosides, contains three hydroxyl groups on contiguous carbon atoms.

Substances exhibiting two reactive sites per molecule. Methyl α-D-galactopyranoside contains four hydroxyl groups, but only the pairs at the 2,3- and 3,4-positions had been thought to be suitably situated for chelation by copper.[4] Since the hydroxyl on carbon 3 is common to both sites this concept required that there should have been only one reactive site per molecule, a conclusion supported by the observation that glycerol reacts with only one atom of copper. But, rotation along the C_5-C_6 bond might place the hydroxyl groups at positions 4 and 6 in a position favorable for chelation thus producing two reactive sites per molecule, and in conformations other than C1 this could occur more readily for a galactoside than for a glucoside. Evidence drawn from the behavior of the methyl D-galactoside indicates that reaction did occur at a second site when relatively concentrated solutions were employed. That the second reaction site involves the 4 and 6 hydroxyls was indicated by the finding of a definite 1:1 reaction between cuprammonium and phenyl 2,3-di-*O*-methyl-β-D-galactopyranoside. Methyl β-L-arabinopyranoside, a substance having configurational and conformational similarity to the D-galactoside, but lacking the terminal carbinol group, gave only the 1:1 reaction.

Di-D-fructopyranose 1,2′-2,1′-dianhydride contains six hydroxyl groups situated in such a way that two reactive sites should exist in the molecule, one on each fructose moiety. The evidence drawn from continuous variation experiments with this substance is illustrated in Fig. 2. Both curves show a break at approximately the 2:1 copper to dianhydride ratio which is in accord with expectations based on the structure of the dianhydride molecule and the idea of a 1:1 copper to diol combining ratio.

Illustrated in Fig. 3 are the results of three different continuous-variation studies carried out to explore the copper to sucrose combining ratio. Curve A shows the results of an experiment employing relatively great copper and sucrose concentrations and highly alkaline conditions. Curve B shows results obtained under less alkaline conditions and at lower copper and sucrose concentrations, while curve C reflects still greater dilution and less alkaline conditions. The break in curve A is indicative of the 2:1 copper to sucrose ratio while the break in curve C is indicative of the 1:1 combining ratio. Curve B clearly reflects the presence of a 1:1 component, but it has asymmetry to the left of the midpoint which signifies the presence of a second component of a higher combining ratio. These findings are interpreted to suggest that sucrose contains two reactive sites for cuprammonium,

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[4] R. E. Reeves, *J. Am. Chem. Soc.*, 71, 1737 (1949).

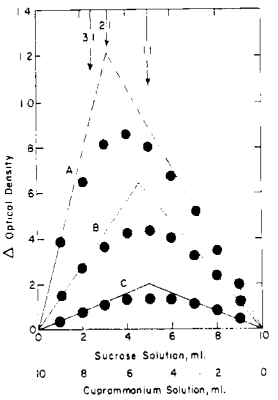

Fig. 3. Continuous-variation experiments between sucrose and cuprammonium.

A. Sucrose $0.092M$, in $11.7M$ ammonia containing $0.184M$ sodium hydroxide *vs.* $0.092M$ cupric nitrate in $11.7M$ ammonia containing $0.184M$ sodium hydroxide 370 mμ

B. Sucrose, $0.04M$, in $6M$ ammonia containing $0.08M$ sodium hydroxide *vs.* $0.04M$ cupric hydroxide in $6M$ ammonia; 360 mμ

C. Sucrose, $0.02M$, in $3M$ ammonia containing $0.04M$ sodium hydroxide *vs.* $0.02M$ cupric nitrate in $3M$ ammonia containing $0.04M$ sodium hydroxide; 360 mμ

one more reactive than the other. Undoubtedly, one site involves a pair of hydroxyl groups on the glucopyranoside ring and the second might bridge between the C_1 hydroxyl of the fructose moiety and the C_2 hydroxyl of the glucose. That both chelate sites do not occur on the D-glucopyranoside ring is indicated by the observation that methyl α-D-glucopyranoside showed no tendency toward a 2:1 combining ratio in equally concentrated solutions. The hydroxyl groups on C_3 and C_4 of the D-fructose moiety, being *trans* on a furanoside ring, would not be expected to chelate with cuprammonium.[5]

The results drawn from studies on a wide variety of diol-containing substances indicate that the cuprammonium-diol combining ratio is 1:1, and that this applies equally to the simple diols and to the glycosides. This conclusion is in accord with that reached in studies on the cuprammonium-mannosan reaction[6] and with studies on copper(II)ethylenediamine-diol chelates.[7,8] However, it is not in accord with the conclusion arrived at by McDonald[3] in a study involving five substances, four of which were also included in the present investigation. This lack of agreement is attributed, in part, to the present finding of two reactive sites on the sucrose and galactoside molecules, and, in part, to the fact that the diol solutions employed by McDonald did not contain alkali equivalent to that present in the cuprammonium solutions.

EXPERIMENTAL

Cuprammonium reagents. Three different methods were employed to prepare the cuprammonium hydroxide reagents used in this study. By one method air was bubbled first through aqueous ammonia and then through a cylinder loosely packed with clean copper turnings and filled with chilled, concentrated aqueous ammonia. In a second method freshly precipitated cupric hydroxide[9] was dissolved in aqueous ammonia; and in a third, sodium hydroxide was added to a solution of cupric nitrate in aqueous ammonia. All three methods produced reagents giving similar results in the continuous variation studies. Upon exposure to air, ammonia is slowly oxidized to nitrite in alkaline cuprammonium solutions and the nitrite ion is strongly absorbing in the near ultraviolet region most useful for the study of the chelation reaction. An appreciable nitrite concentration is unavoidable in cuprammonium prepared by the first method which is, for this reason, the least satisfactory.

Physical observations. Observations were made at room temperature which was 27° ± 2°. The potentiometric titrations were made with a Beckman Model G *p*H meter equipped with external calomel and Type E glass electrodes. The reference solution contained $0.1M$ ammonium nitrate and $0.2M$ ammonia in water for which the *p*H value 9.55 was taken.

Optical densities were determined in a Beckman Model DU spectrophotometer equipped with 1-cm. cells. Observations were made within a few moments after making up the solutions in order to keep to a minimum the formation of nitrite in the solutions. Most observations were made at 360 mμ, however, in a few experiments a longer wavelength was chosen in order to reduce the densities to values which could be more accurately determined by the instrument.

Optical rotations were observed visually in a Schmidt and Haensch polarimeter using 0.5-dm. cells The light source was a G.E. H-4 lamp backed by a plane mirror in a standard ventillated housing. Corning filters 3389 (3 mm.) and 5113 (2 mm.) were used to isolate the mercury 436 mμ line.

Erythritol anhydride was prepared by the method of Henninger.[10] Assay by lead tetraacetate titration[11] showed it to contain 99% of the theoretical amount of *cis* hydroxy groups. Its acid dissociation constant, determined by the method of Michaelis[12] was found to be 8×10^{-14}; pK_a 13.1.

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(5) R. E. Reeves, *J. Am. Chem. Soc.*, **71**, 212 (1949).

(6) R. E. Reeves, *J. Am. Chem. Soc.*, **73**, 957 (1951).

(7) H. B. Jonassen, R. E. Reeves, and L. Segal, *J. Am. Chem. Soc.*, **77**, 2667 (1955).

(8) L. Segal, H. B. Jonassen, and R. E. Reeves, *J. Am. Chem. Soc.*, **78**, 273 (1956).

(9) To an aqueous solution of cupric chloride was added just sufficient aqueous ammonia to precipitate and then redissolve the cupric hydroxide. To this solution was added an excess of carbon dioxide-free sodium hydroxide to re-precipitate the cupric hydroxide which was then washed by centrifugation with cold carbon dioxide-free water until free from chloride ion. The moist cupric hydroxide was then immediately dissolved in aqueous ammonia.

(10) Henninger, *Ann. Chim. Phys.* (**6**) **7**, 223 (1886).

(11) R. E. Reeves, *Anal. Chem.*, **21**, 751 (1949).

(12) L. Michaelis, *Ber.*, **46**, 3683 (1913).