of two other highly branched hydrocarbons, 2,2-dimethylpropane (4300 cal. mole^{-1})[16] and 2,2-dimethylbutane (4375 cal. mole^{-1}).[17]

Thermodynamic Functions.—The functions $-(F^0 - H_0^0)/T$, $H^0 - H_0^0$, S^0 and C_p^0 for 2,2,3,3-tetramethylbutane were computed for selected temperatures up to 1500°K., using the vibrational assignment, moments and reduced moments of inertia, and value of the average barrier height discussed in previous sections. The 1949 atomic weights and the values of the fundamental constants given by Wagman, *et al.*,[18] were used in all computations of this paper. The calculated thermodynamic functions are presented in Table VI. For reasons of internal consistency, all tabulated values are given to four digits, although in some places in the table the retention of the last digit is not justified by the accuracy of the molecular-structure parameters used in the calculations or by the reliability of the approximations employed.

TABLE VI

THERMODYNAMIC FUNCTIONS OF 2,2,3,3-TETRAMETHYLBUTANE

T, °K.	$-(F^0 - H_0^0)/T$, cal. deg.$^{-1}$ mole^{-1}	$H^0 - H_0^0$ kcal. mole^{-1}	S^0, cal. deg.$^{-1}$ mole^{-1}	C_p^0, cal. deg.$^{-1}$ mole^{-1}
298.16	67.97	7.482	93.06	46.03
300	68.12	7.565	93.34	46.29
400	76.33	12.88	108.5	59.88
500	84.27	19.48	123.2	71.76
600	91.94	27.17	137.2	81.52
700	99.35	35.73	150.4	89.52
800	106.5	45.02	162.8	96.18
900	113.4	54.92	174.4	101.8
1000	120.1	65.34	185.4	106.6
1100	126.5	76.21	195.8	110.8
1200	132.7	87.49	205.6	114.4
1300	138.7	99.04	214.9	117.5
1400	144.4	110.9	223.7	120.1
1500	150.0	123.1	232.0	122.5

(16) K. S. Pitzer and J. E. Kilpatrick, *Chem. Revs.*, **39**, 435 (1946).

(17) J. E. Kilpatrick and K. S. Pitzer, THIS JOURNAL, **68**, 1066 (1946).

(18) D. D. Wagman, J. E. Kilpatrick, W. J. Taylor, K. S. Pitzer and F. D. Rossini, *J. Research Natl. Bur. Standards*, **34**, 143 (1945).

BARTLESVILLE, OKLAHOMA RECEIVED JULY 17, 1951

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Thermodynamics of Metallo-Protein Combinations. Comparison of Copper Complexes with Natural Proteins

BY HAROLD A. FIESS AND IRVING M. KLOTZ

The affinity of each of five proteins for cupric ion in acetate buffer at *p*H 6.5 has been found to decrease in the following order: α-casein > β-casein > bovine serum albumin > β-lactoglobulin > lysozyme. Relative affinity for copper parallels the isoelectric point of these proteins. The variation in binding with copper concentration is less than might be expected on superficial examination of the electrostatic factors involved, but more careful consideration indicates some reasons for the discrepancy. Binding of copper by α-casein exhibits no temperature dependence between 0° and 25°. Furthermore heating of the protein to 90° for 1 hour does not reduce its affinity for copper. These characteristics, as well as the interactions of copper with the polypeptides of glutamic acid and of lysine, emphasize the importance of electrostatic factors in the formation of copper–protein complexes.

Introduction

Detailed thermodynamic studies of copper complexes with crystallized bovine serum albumin have been reported recently.[1,2] In these papers the effects of environmental factors, such as *p*H, type of buffer, and temperature, on the stability of copper–albumin complexes have been described. Further investigations comparing the affinities for copper of a number of native proteins and related polypeptides are described in the present paper.

Experimental

Quantitative binding studies were made by the equilibrium–dialysis technique described previously,[1] except that smaller volumes of solution were used.[3] The protein concentration was 0.5% in all binding experiments.

Analyses for cupric ion concentrations were made by the cuprethol method of Woelfel.[4] This method gave accurate results for copper in the presence of acetate, citrate, phosphate or borate ions as well as in the presence of any of the proteins used in this study. An acetate buffer of *p*H 5, as recommended by Woelfel, was used in most analyses. In the analysis of casein-containing solutions, however, precipitation occurred at this *p*H and therefore an acetate or citrate buffer of *p*H 6.5 was used.

Absorption spectra were obtained with a Beckman spectrophotometer, model DU, using one-centimeter cells. Molecular extinction coefficients, ε, were calculated from the equation

$$\log_{10} \frac{I_0}{I} = \epsilon c d$$

where I_0 is the intensity of the light emerging from the solvent, I is the intensity of the light emerging from the solution, c is the molar concentration of solute, and d is the thickness of the absorption cell in centimeters.

Crystallized bovine serum albumin and lysozyme were obtained from Armour and Co. Samples of crystallized β-lactoglobulin were kindly supplied by Dr. L. Lachat of Armour and Co. The lactoglobulin gave signs of the presence of trace metals in blank tests with cuprethol reagent. This protein was dialyzed, therefore, against 0.02 *M* sodium citrate to remove the trace metals and then against water to remove the citrate. The purified protein was lyophilized.

α-Casein and β-casein were prepared from unpasteurized skim milk by the procedure of Warner.[5] All-glass equipment was used to prevent contamination by trace metals. The fractionated caseins gave electrophoretic patterns similar to those described by Warner.[5]

A sample of natural polyglutamic acid was kindly supplied by Dr. H. Fraenkel-Conrat. Polylysine hydroiodide was a gift from Dr. E. Katchalski.

(1) I. M. Klotz and H. G. Curme, THIS JOURNAL, **70**, 939 (1948).

(2) I. M. Klotz and H. A. Fiess, *J. Phys. Colloid Chem.*, **55**, 101 (1951).

(3) I. M. Klotz, J. M. Urquhart and W. W. Weber, *Arch. Biochem.*, **26**, 420 (1950).

(4) W. C. Woelfel, *Anal. Chem.*, **20**, 722 (1948).

(5) R. C. Warner, THIS JOURNAL, **66**, 1725 (1944).

A reagent grade sample of $CuCl_2 \cdot 2H_2O$ served as the source of cupric ion. Buffers were prepared from salts of reagent grade or equivalent quality.

Results and Discussion

Affinities of Proteins for Copper in Acetate Solution.—The extent of binding of cupric ions by each of a series of proteins is illustrated in Fig. 1. The degree of binding is represented in graphs of the average number of bound ions per 10^5 grams of protein *versus* the logarithm of the concentration of unbound cupric ion [labeled $(copper)_{free}$]. All of the data in Fig. 1 were obtained with an acetate buffer of *p*H 6.5. A relatively high concentration of acetate, 0.2 *M*, was needed to keep cupric ions in solution at this high *p*H. It is immediately evident from Fig. 1 that each of the proteins examined, except lysozyme, is capable of binding large quantities of copper.

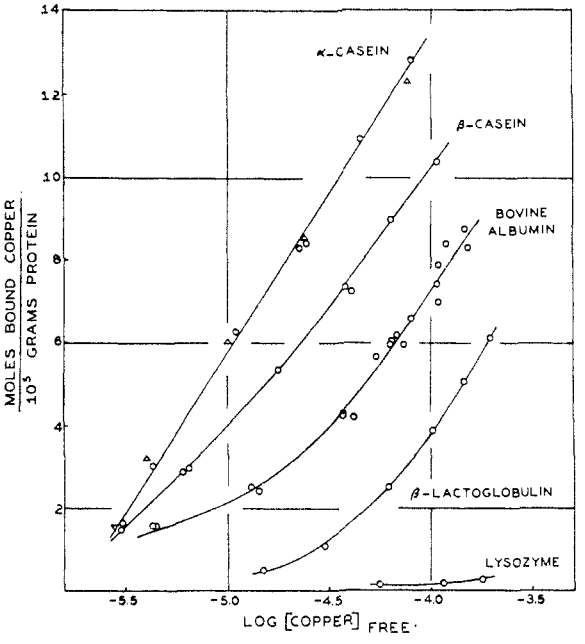

Fig. 1.—The extent of binding of cupric ions in acetate solution, *p*H 6.5, by several proteins. For α-casein, O refers to 0°, Δ to 25°.

The application of the law of mass action to equilibria of this type has been described in detail previously.[1] The formation of a series of complexes between copper and a given protein, P, may be represented by the equation

$$PCu_{i-1} + Cu^{++} = PCu_i \qquad (1)$$

where PCu_{i-1} and PCu_i indicate complexes with $i - 1$ and i cupric ions, respectively. If the binding sites all have the same intrinsic binding constant k, then, as has been shown earlier[6]

$$\lim_{(Cu^{++}) \to 0} \left[\frac{r}{(Cu^{++})}\right] = nk \qquad (2)$$

where r is the moles of bound ion per mole of total protein and n is the total number of available sites. For a single set of binding sites, furthermore

$$nk = k_1 \qquad (3)$$

where k_1 is the equilibrium constant for the first copper complexed with the protein. Thus k_1 can also be calculated from an extrapolation of the experimental binding data and hence ΔF_1° may be computed from the general thermodynamic relation

$$\Delta F_1^\circ = -RT \ln k_1 \qquad (4)$$

Values of ΔF_1° for each of the five proteins in acetate buffer are listed in Table I. The relative affinities for Cu^{++} decrease in the order α-casein > β-casein > bovine albumin > β-lactoglobulin > lysozyme.

TABLE I

FREE ENERGIES OF BINDING OF CUPRIC ION BY PROTEINS[a]

0°, *p*H 6.5

Protein	ΔF_1°, kcal. mole^{-1} In 0.2 *M* acetate buffer	In 0.01 *M* citrate buffer	Isoelectric *p*H
α-Casein	−7.30	−5.22	4.0[b]
β-Casein	−7.16	−5.91	4.5[b]
Bovine serum albumin	−6.49	−5.71	4.71[c]
β-Lactoglobulin	−5.81	....	5.21[c]
Lysozyme	−4.39	....	11.0[d]

[a] For purposes of comparison, a unit weight of 100,000 was used for each protein. [b] In 0.1 μ acetate solution as given by R. C. Warner, THIS JOURNAL, 66, 1725 (1944). [c] In 0.1 μ acetate solution as given by L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, 53, 126 (1949). [d] In 0.1 μ buffer as given by G. Alderton, W. H. Ward and H. L. Fevold, *J. Biol. Chem.*, 157, 43 (1945).

The value listed for lysozyme is less reliable than the values for the other proteins because some lysozyme leaks through the cellophane bag, apparently due to the protein's low molecular weight (*ca.* 14,000).[7] A set of separate experiments indicates that over a 24-hour period this leakage may be as high as 20% of the total protein inside the bag. Equilibrations in binding experiments never were longer than 24 hours.

Comparison of Spectra of Copper–Protein Complexes.—It is of interest to note that the spectra of the copper complexes of these proteins indicate an order of affinity similar to that derived from

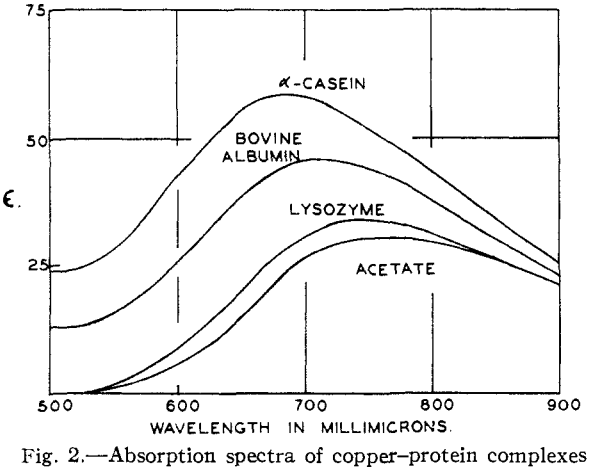

Fig. 2.—Absorption spectra of copper–protein complexes in 0.2 *M* acetate solution at *p*H 6.5. Protein concentrations: casein, 1%; albumin, 2%; lysozyme, 2%. Copper concentration: with casein, 0.002 *M*; in all other solutions, 0.005 *M*.

(6) I. M. Klotz and J. M. Urquhart, *J. Phys. Colloid Chem.*, 53, 100 (1949).

(7) K. J. Palmer, M. Ballantyne and J. A. Galvin, THIS JOURNAL, 70, 906 (1948).

equilibrium dialysis experiments. Absorption curves are illustrated in Fig. 2. An increase in extinction coefficient and a shift of the absorption maximum toward shorter wave lengths is indicative of increased binding affinity.[2] The arrangement of α-casein, bovine albumin and lysozyme on the basis of the optical properties of their copper complexes parallels the order obtained for binding ability from dialysis measurements.

Relative Affinity for Copper and Molecular Character of Protein.—The question immediately arises, therefore, whether the order of binding ability of these proteins may be related to any fundamental molecular characteristics of these substances. Attempts to correlate binding affinity with the number of anionic sites in these molecules have not proved fruitful. Bovine albumin, for example, contains more carboxyl groups than does β-casein, yet binds copper less. Even if account is taken of the number of phosphate groups in β-casein, the total equivalents of anionic sites do not equal those in albumin. Similarly, although albumin contains fewer carboxyl groups than β-lactoglobulin, albumin has a greater avidity for copper. It must be conceded, however, that the two caseins, containing phosphate groups in contrast to the other proteins, form stronger copper complexes and their affinities parallel phosphate content.

Similar comparisons of the number of lysine and histidine groups with copper uptake have also revealed no parallel.

On the other hand, a very definite correlation for all five proteins seems to be present between binding affinity in acetate buffer and isoelectric point. Values of the isoelectric pH's at 0.1 μ given in the literature have been presented in Table I.

Variation in Binding with Concentration of Copper.—The dependence of extent of binding on the concentration of free cupric ion is also of interest in the study of these metallo-proteins. It has been found earlier[1] with serum albumin at pH 4.8 that the dependence of binding on copper concentration can be described quantitatively in terms of a set of binding sites with a single intrinsic binding constant plus a suitable correction for electrostatic interactions. The electrostatic interaction term which agreed best with the experimental observations, however, was markedly smaller than would be expected from application of the Debye–Hückel theory.

An analysis of the binding data given in the present paper leads to similar conclusions. The extent of binding can be correlated satisfactorily in terms of a single set of binding sites with a suitable term for electrostatic interactions. A value for the electrostatic interaction term which best fits the experimental data was obtained by application of one of the binding equations suggested by Scatchard[8]

$$\ln \frac{r}{(n-r)(\mathrm{Cu}^{++})} = \ln k - 2wr \qquad (5)$$

In this equation w represents an electrostatic factor which is related to the electrostatic free-energy term used previously[1] by the expression

$$\Delta F_{\mathrm{elec}} = 2wRT \qquad (6)$$

where, as usual, R represents the gas law constant and T the absolute temperature. The quantity ΔF_{elec} of equation (6) is the electrostatic portion of the free-energy difference, $\Delta F_i^\circ - \Delta F_{i-1}^\circ$, between the reactions

$$\mathrm{PCu}_{i-2} + \mathrm{Cu}^{++} = \mathrm{PCu}_{i-1}; \quad \Delta F_{i-1}^\circ \qquad (7)$$

and

$$\mathrm{PCu}_{i-1} + \mathrm{Cu}^{++} = \mathrm{PCu}_i; \quad \Delta F_i^\circ \qquad (8)$$

The value for w obtained with equation (5) depends on the value assumed for n. It was not possible to carry out a reliable extrapolation for n within the limits of copper concentration attainable in 0.2 M acetate. Fortunately, however, w is not very sensitive to the assumed value for n and varies by only about 30% if n is changed from 20 to 100. Subject to these uncertainties, a ΔF_{elec} of approximately 25 calories was calculated from the experimental data for serum albumin. From theoretical considerations it can be shown[1] that

$$\Delta F_{\mathrm{elec}} = \frac{Nz^2e^2}{D}\left[\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right] \qquad (9)$$

where N is Avogadro's number, z the number of charges on the small ion, e the electronic charge, D the dielectric constant of the medium, b the radius of the protein molecule, a the distance of closest approach of a small ion to the protein and κ the well-known factor in Debye–Hückel theory which contains the ionic strength. For bovine serum albumin b has been taken as 30 Å. The theoretical value of ΔF_{elec} thus becomes 125 calories, clearly much larger than that required by the experimental data. The discrepancy is in the same direction as, though somewhat larger than, that observed at pH 4.8.[1]

There are at least two reasons why ΔF_{elec} calculated from equation (9) may be too large. In the first place, the combination of the protein with Cu^{++} ions must result in the displacement of some H^+ ions because of electrostatic factors as well as because both small cations are competing for a common type of binding site. In addition, it must not be overlooked that the experiments were carried out in acetate buffer, in which a large portion of the cupric ion is in the form[9] $Cu(C_2H_3O_2)^+$. Since there is evidence that the latter species is bound as such by the protein,[2] the actual increase in charge of the complex is less than would occur on the binding solely of Cu^{++} ions. Both of these factors would reduce the electrostatic repulsion term due to successively bound ions.

The displacement of H^+ ions by bound Cu^{++} is not evident in solutions containing acetate since the latter acts as a pH buffer. Changes in pH are readily evident, however, in the absence of buffer other than the protein. For example, in Fig. 3, the pH's of isoionic albumin in solutions of various concentrations of Cu^{++} are shown, and it is quite apparent that H^+ ions are released by the protein when it binds Cu^{++}. In the absence of protein, the pH of a 0.1 M $CuCl_2$ solution is approximately 0.5 unit higher than any pH shown in Fig. 3.

(8) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

(9) K. J. Pedersen, *Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd.*, **22**, No. 12 (1945).

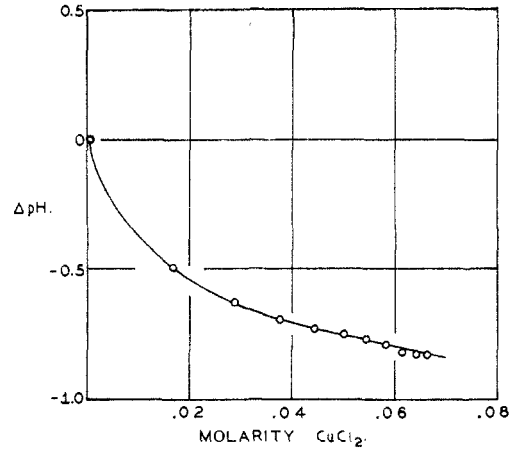

Fig. 3.—Change in *p*H of isoionic bovine serum albumin in aqueous 0.1 *M* sodium chloride solution upon addition of cupric chloride.

Variation in Binding with Temperature.—The effect of temperature on the binding of copper by serum albumin has been reported previously.[1,2] Increasing temperature produced only a slight increase in binding by this protein. Similar results have been observed in an examination of α-casein in the present investigation, no significant change in uptake of Cu^{++} being obtained as the temperature is increased from 0 to 25° (Fig. 1). Once again it is clear that the enthalpy change for the binding process is essentially zero. Consequently, the entropy change, which may be computed readily from the equation

$$\Delta S_1^\circ = -\frac{\Delta F_1^\circ}{T} \qquad (10)$$

is approximately 27 calories $mole^{-1}$ $degree^{-1}$. The significance of large positive entropy changes in binding processes has been discussed previously.[1,10]

Affinities of Proteins for Copper in Citrate Solution.—In citrate buffer, binding of copper by each of the proteins (Fig. 4) is reduced considerably as compared to the uptake in acetate solution. Citrate ions of course form much stronger complexes[11,12] with Cu^{++} than do acetate ions[9] and hence they reduce the concentration of free Cu^{++} substantially. On the other hand, the reduction in binding does not seem nearly as great as one might expect from the decrease in concentration of non-complexed Cu^{++}.

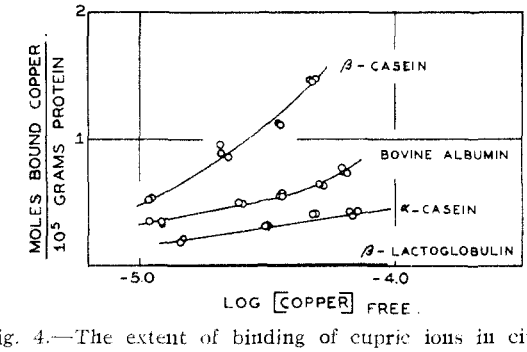

Fig. 4.—The extent of binding of cupric ions in citrate solution, *p*H 6.5, by several proteins.

It is known, however, that anions such as citrate are also bound by serum albumin. Unpublished experiments in this Laboratory show that the caseins also bind anions. It seems possible, therefore, that some uptake of ions such as $CuC_6H_5O_7^-$ (and perhaps $CuC_6H_4O_7^=$)[11] may also occur, localized, presumably, at the basic-amino-acid side-chains of the protein. Furthermore, the binding of buffer ions themselves, $C_6H_5O_7^=$ and $C_6H_6O_7^=$, would facilitate uptake of simple Cu^{++} ions by a coöperative electrostatic effect. For all three proteins, therefore, the extent of binding of cupric ions in citrate buffer may be greater than would be anticipated from the non-complexed Cu^{++} concentration.

Binding energies for copper in citrate buffer are listed in Table I. A comparison of the order of the proteins in acetate (Fig. 1) and citrate (Fig. 4) buffers reveals an unanticipated discrepancy. In each buffer the following order of affinity for copper prevails: β-casein > albumin > β-lactoglobulin. However, in acetate solutions α-casein exceeds β-casein in affinity for copper, whereas in citrate buffers α-casein falls not only below β-casein but also substantially below serum albumin. It is apparent that citrate buffer has some special effect on α-casein.

Effect of Heating Protein.—In contrast to anion complexes, the complexes of protein with copper do not seem to be disturbed by exposing the protein solution to temperatures near 100°. A set of experiments was carried out in this connection with α-casein. A solution of 0.5% α-casein and approximately 5×10^{-4} *M* copper chloride in 0.2 *M* acetate at *p*H 6.5 was heated at 90° for one hour. At the end of the heating period, the extent of binding at 0° was determined. Experiments were also carried out in which glycine, glutamic acid and urea, respectively, in concentrations at which they might be encountered in milk, were added to the protein solution before heating. In all cases, the extent of binding of the heated protein did not differ significantly from that of the unheated material.

Thus we have a further indication that the binding of Cu^{++} by proteins is not especially sensitive to configuration with the macromolecule. Evidently Cu^{++} can form complexes with the side-chains with such great avidity that minor perturbations in their arrangement are not critical.

Interaction of Copper with Polar Polypeptides.—The interactions of copper with natural polyglutamic acid have also been investigated. In this case a precipitate of copper polyglutamate was formed. The extent of uptake of Cu^{++} was determined from the depletion of free copper in the medium. Precipitation started at a free copper concentration near 2×10^{-3} *M* and the extent of copper binding rose rapidly to a pleateau at a free copper concentration near 8×10^{-3} *M*. The extent of binding in this region corresponds to one mole of copper for every two glutamic acid residues. Since each residue contains only one free carboxyl group, the other being involved in the peptide

(10) I. M. Klotz and J. M. Urquhart, THIS JOURNAL, **71**, 847 (1949).
(11) M. Bobtelsky and J. Jordan, *ibid.*, **67**, 1824 (1945).
(12) L. Meites, *ibid.*, **72**, 180 (1950).

linkage, the maximum quantity of bound copper serves to balance the free carboxyl groups in the expected ratio of 1:2.

A few spectrophotometric experiments were also carried out with 0.01 *M* copper in aqueous 0.1 *M* NaCl solutions of synthetic polylysine at a *p*H of 4.9. The spectra obtained could not be distinguished from those of copper chloride in 0.1 *M* NaCl aqueous solution. In this respect polylysine behaved like lysozyme. Evidently the large positive charge on the polypeptide molecule produces such a strong repulsion toward Cu^{++} ions that even at a concentration of 1×10^{-2} *M* they are unable to compete effectively with 1×10^{-4} *M* hydrogen ions for the amine groups.

Conclusions.—In contrast to protein interactions with anions, the interactions described in the present paper indicate no unique affinity characteristic of any one of the proteins examined. Binding of cupric ions thus seems to be more nearly analogous to uptake of hydrogen ions by proteins. The requirements for binding seem to be primarily the presence of appropriate residues and favorable electrostatic conditions in the macromolecule.

Acknowledgment.—This investigation was supported by grants from the Carnation Company. We are indebted especially to Dr. E. B. Oberg and Mr. W. C. Cross for their interest.

EVANSTON, ILLINOIS RECEIVED JUNE 8, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING AT THE UNIVERSITY OF WASHINGTON]

Structural Properties of Chromium(III) Iodide and Some Chromium(III) Mixed Halides

BY L. L. HANDY AND N. W. GREGORY

Powder patterns indicate that chromium(III) iodide is isostructural with chromium(III) chloride, with hexagonal unit cell parameters $a = 6.86$ and $c = 19.88$. The three mixed halides $CrICl_2$, $CrIBr_2$ and $CrBrCl_2$, formed by reaction of the less active halogen with the corresponding chromium(II) salt, appear structurally equivalent to that expected for an ideal solid solution of CrX_3 and CrY_3, with the exception that the halide layers are not as closely packed.

The crystal structures of anhydrous chromium(III) iodide and of some chromium(III) mixed halides ($CrICl_2$, $CrIBr_2$ and $CrBrCl_2$) have been investigated. The products indicated by the formulas $CrXY_2$ were formed by reaction of the less active halogen X_2 with the chromium(II) salt CrY_2. The structural relationship of the mixed and simple halides was of particular interest. A report of some thermodynamic properties of these substances will be made in a forthcoming paper.

The structures of the chromium(III) halides are sufficiently similar to permit indexing of powder patterns from the known characteristics of $CrCl_3$ and $CrBr_3$. Wooster[1] has made a detailed study of the anhydrous chromium(III) chloride structure. It has been assigned the space group $D_3^3(H3_12)$-D_3^5 ($H3_22$) with $a = 6.02$ Å. and $c = 17.3$ Å. Braekken[2] found $CrBr_3$ to have a very similar structure with slightly different space group, C_3^1 (C3) or C_{3i}^1 ($C\bar{3}$) with $a = 6.26$ Å. and $c = 18.2$ Å. A large group of metallic halides with formula MX_3 crystallize with the latter arrangement, frequently called the BiI_3 structure, but the $CrCl_3$ arrangement has not hitherto been found among other compounds of this type. Both structures consist of a double-layer lattice of halogen atoms based on a closest packing arrangement (cubic in $CrCl_3$ and hexagonal in $CrBr_3$). Chromium atoms in a regular hexagonal arrangement occupy two-thirds of the octahedral interstices within the double layers, but different double layers are apparently held together by van der Waals forces only.

The structure factors are such as to permit an easy distinction between the two types: diffraction lines from the 112, 114, 118, etc., are characteristic of $CrCl_3$, whereas 113, 116, 119, etc., appear from $CrBr_3$. In the present work the powder patterns of CrI_3 and of the mixed halides prepared by halogen oxidation correspond to the $CrCl_3$ structure.

Experimental Part

Powder patterns were taken with samples in sealed thin walled Pyrex capillary tubes approximately 0.6 mm. in diameter. The sample tubes were prepared in a dry-box. Exposures were made for 24- to 36-hour period, using copper radiation filtered with nickel. Several samples from independent preparations of each material were used with satisfactory agreement of spacings observed on each. The values reported were taken from the best pattern in each case.

The preparation of CrI_3 has been described in an earlier paper.[3] Anhydrous $CrBr_3$ and $CrCl_3$ were prepared by standard methods and purified by sublimation under high vacuum in quartz. The mixed halides $CrIBr_2$ and $CrICl_2$ were prepared by reaction of iodine with $CrBr_2$ and $CrCl_2$, respectively, in a manner analogous to that described for iodination of CrI_2.[3] The decomposition pressure of iodine above these substances becomes quite large before the vapor pressure is sufficiently high to effect sublimation. The reaction of iodine with the chromium(II) salts was observed to be reversible.

$CrBrCl_2$ was prepared in a similar manner by bromination of $CrCl_2$. Other mixtures of varying composition in the $CrCl_3$–$CrBr_3$ system were prepared by heating predetermined amounts of $CrCl_3$ and $CrBr_3$ in high vacuum until sublimation occurred ($\approx 700°$). On condensation of the vapor a homogeneous product was obtained.

Analyses.—The treatment necessary to dissolve anhydrous chromium(III) halides makes good halogen determinations difficult. On ignition in air these compounds liberate halogen and form Cr_2O_3. This has been found satisfactory as a method of determining halogen content by weight loss. The oxide may be fused with sodium peroxide and the chromium content confirmed by standard analytical methods.

Since either oxide impurity or an excess of chromium(II) salt from incomplete reaction would make the chromium percentage appreciably higher than the theoretical value expected for the mixed halide, it was considered sufficient

(1) Nora Wooster, *Z. Krist.*, **74**, 363 (1930).

(2) H. Braekken, *Kgl. Norski. Videnskab. Selskab. Forh.*, **5**, No. 11 (1932).

(3) L. L. Handy and N. W. Gregory, THIS JOURNAL, **72**, 5049 (1950).