[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MICHIGAN]

Thermodynamics of Chelation. I. The Statistical Factor in Chelate Ring Formation[1]

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The relative contributions of entropy and enthalpy terms to the stability of chelate complexes of Cd^{++} have been determined by comparing the thermodynamic functions for the methylamine, ethylenediamine and ammonia complex ions of cadmium(II) over the temperature range of 10 to 40°. The increase in the stability of cadmium chelates is purely an entropy effect. It has also been noted that the thermodynamic functions for $Cd(NH_3)_2^{++}$ and $Cd(NH_2Me)_2^{++}$ are almost identical.

The increase in the stability of coördination compounds which results from chelate ring formation is widely recognized. This enhanced stability of chelate systems has been attributed both to an increase in bond strength resulting from formation of the ring[2] and to a statistical or probability effect associated with chelation.[3] Although several previous investigators[4-6] have mentioned these factors in chelation, no systematic attempt to evaluate the relative contribution of each has been recorded. Such a study is described herein.

Since the enthalpy of formation, ΔH, is considered a measure of the binding energy,[7] and the entropy of formation, ΔS, includes all probability factors controlling the stability of the complex, measurement of these thermodynamic functions should provide the desired information.

Since the ethylenediamine molecule is the equivalent of two methylamine molecules joined together by the elimination of two hydrogen atoms, a chelate ethylenediamine complex might well be compared with a corresponding non-chelate bismethylamine complex of the same metal cation. Such a comparison minimizes complications of interpretation such as those due to hydration energies and electronic effects resulting from the methylene group. Uncertainties resulting from the secondary influences of one coördinated group upon another can also be minimized by restricting the comparison to the monoethylenediamine and bismethylamine complex ions. The reactions studied were

Non-chelate

$$[M(H_2O)_n]_{(aq)}^{++} + 2NH_2CH_{3(aq)} \rightleftarrows [M(H_2O)_{n-2}(CH_3NH_2)_2]^{++}_{(aq)} + 2H_2O$$

Chelate

$$[M(H_2O)_n]_{(aq)}^{++} + en_{(aq)} \rightleftarrows [M(H_2O)_{n-2}(en)]_{(aq)}^{++} + 2H_2O$$

The thermodynamic functions ΔH, ΔF and ΔS were obtained from measurements of the equilibrium constants over the temperature range of 10 to 40°, at 5° intervals. The familiar method of Bjerrum[2] was used to determine the concentration equilibrium constants.

The choice of the metal ion for the present investigation was limited very severely by the fact that the greater base strength and lower coördinating power of methylamine caused most metal ions to precipitate from solution as the metal hydroxide before soluble coördination compounds containing two methylamine molecules would form. Of the metal ions tested (Cd^{++}, Zn^{++}, Cu^{++}, Ni^{++}, Co^{++}), cadmium(II) was the only cation which could be used satisfactorily in the present measurements.

In order to determine the magnitude of the effects resulting from the presence of the methyl group on the nitrogen, constants from 10 to 40° were also obtained for the reaction involving ammonia.

$$[Cd(H_2O)_n]_{(aq)}^{++} + 2NH_{3(aq)} \rightleftarrows [Cd(H_2O)_{n-2}(NH_3)_2]_{(aq)}^{++} + 2H_2O$$

Experimental

A. **Reagents.** 1. **Methylamine.**—Methylamine hydrochloride was purified by the method of Brown and Bartholomay.[8] The methylamine was distilled into freshly boiled distilled water from a solution of methylamine hydrochloride to which excess KOH had been added. The distillation was carried out in a CO_2-free atmosphere.

2. **Methylammonium Nitrate.**—Methylammonium nitrate solution was prepared by distilling methylamine directly into HNO_3 solution.

3. **Ammonia.**—Reagent grade ammonium hydroxide was redistilled from KOH solution in the same manner as the methylamine.

4. **Ethylenediamine.**—Eastman Kodak 95–100% ethylenediamine was redistilled in an all glass apparatus collecting the fraction boiling at 115 to 116°. Each of the amine reagents was stored in an all glass container fitted with a siphon delivery tube and an ascarite filled drying tube.

5. **Other Reagents.**—Reagent grade NH_4NO_3, $Ba(NO_3)_2$, KNO_3 and $Cd(NO_3)_2$ were used without further purification. An approximately 3 *M* stock solution of $Cd(NO_3)_2$ was prepared and carefully standardized by electrodeposition of Cd from an alkaline cyanide solution. Weighed aliquots of the stock solution were used in preparing the working solutions.

6. **Working Solutions.**—Two liters of each of the solutions indicated in Table I were prepared using stock solutions and freshly boiled redistilled water. The free acid and amine salt content of each solution were carefully determined.

TABLE I

WORKING SOLUTIONS FOR TITRATIONS

Ingredient	Solution (molar concentration) A	B	C	D	E	F
$Ba(NO_3)_2$	0.05	0.05	0.05	..	..	..
HNO_3	0.20	0.20	0.20	0.20	0.20	0.20
NH_4NO_3	1.80	..	..	1.80	..	..
$CH_3NH_3NO_3$	..	1.80	..	..	1.80	..
KNO_3	..	..	1.80	..	..	1.80
$Cd(NO_3)_2$	..	..	..	0.05	0.05	0.05

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[1] Abstracted in part from a thesis presented by Clark G. Spike to the graduate faculty of the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1952.

[2] J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

[3] (a) N. V. Sidgwick, *J. Chem. Soc.*, 433 (1941); (b) A. R. Burkin, *Quart. Rev.*, **V**, 1 (1951).

[4] J. Bjerrum and E. J. Nielsen, *Acta Chem. Scand.*, **2**, 297 (1948).

[5] M. Calvin and R. H. Bailes, THIS JOURNAL, **68**, 953 (1946).

[6] M. Calvin and N. C. Melchior, *ibid.*, **70**, 3270 (1948).

[7] The term binding energy as used here includes all solvation energy terms as well as the usually implied energy of coördination.

[8] H. C. Brown and H. Bartholomay, Jr., THIS JOURNAL, **66**, 435 (1944).

For determination of the acid dissociation constants of ammonia, methylamine, and ethylenediamine, solutions A, B and C, respectively, were used. The metal ammine dissociation constants were determined using solutions D, E and F. In studying the complexes of methylamine and ammonia it was necessary to use a high concentration of the amine salt to prevent precipitation of the metal hydroxide. In order to maintain a constant ionic strength for all determinations, equivalent amounts of KNO_3 were added to solutions C and F which were used for the ethylenediamine determinations. The use of $Ba(NO_3)_2$ to maintain a constant concentration of divalent ions follows a suggestion of Bjerrum.[2]

B. **Procedure.**—The titration vessel was made from a 500-ml. Florence flask fitted with four additional necks made from the tops of 18 mm. test-tubes. These small necks were used to admit the glass electrode, calomel electrode and micro-buret, and as an inlet for CO_2-free nitrogen. A variable speed mechanical stirrer was admitted through the large neck of the flask. The flask was immersed in a constant temperature bath which was maintained at ±0.05°. A 10-ml. micro-buret, equipped with a capillary tip which dipped just below the surface of the liquid, was used for the addition of the amine. One hundred milliliters of the solution to be studied was placed in the titration vessel and the air above the solution was swept out with CO_2-free nitrogen. After allowing 15 to 30 minutes for temperature equilibrium to be reached, the solution was titrated with the amine. A Beckman model F *p*H meter was used with a model 1190–90 glass electrode and a nine-inch saturated calomel electrode. The *p*H meter had been previously standardized against 0.05 *M* potassium acid phthalate buffer. This standardization was carried out in the titration vessel using 100 ml. of buffer solution. At least a half hour was allowed for temperature equilibrium before each *p*H reading on the buffer.

The first portion of the titration involves merely a neutralization of the excess nitric acid present, the end-point of which serves to standardize the amine reagent. Although chemical equilibrium is reached very rapidly, at least two minutes were allowed between addition of the amine and measurement of the *p*H in order to permit thorough stirring and temperature equilibrium. Tests were carried out to determine constancy of *p*H readings with time; the *p*H became constant 30 seconds after the addition of the amine to the metal ion and remained constant for 3 days. Titrations were carried out at 5° intervals from 10 to 40°.

C. **Calculations.**—The approximate constants for the cadmium complexes were obtained directly from the formation curves by the method of Bjerrum.[2] Since these temporary values are in error if appreciable overlapping of constants occurs, the true pK values were obtained from the approximate values by a method of successive approximations similar to that described by Bjerrum. The reliability of the constants is indicated in Fig. 1 by the close agreement between the experimental formation curve for cadmium ammines at 25° and the theoretical curve calculated from the corrected constants. The theoretical curve calculated from the uncorrected constants is also shown for comparison.

The approximation of the consecutive dissociation constants, K_n, is based upon the assumption that $[MA_n] = [MA_{n-1}]$ when $\bar{n} = n - 0.5$, where $\bar{n}$ represents the average number of amine molecules bound per metal ion and is defined by the equation

$$\bar{n} = \frac{[MA] + 2[MA_2] + \cdots n[MA_n]}{[M] + [MA] + [MA_2] + \cdots [MA_n]}.$$

The square brackets represent molar concentrations, and n is the number of ligands about the fully coördinated metal ion.

As an example, for the first consecutive constant for the cadmium–methylamine system

$$k_1 = [A][Cd^{++}]/[CdA^{++}]$$

$[Cd^{++}]$ is assumed to be equal to $[CdA^{++}]$ at $\bar{n} = 0.5$. However, from the defining equation of $\bar{n}$

$$[Cd^{++}] = [CdA^{++}] + 3[CdA_2^{++}] + 5[CdA_3^{++}] + 7[CdA_4^{++}]$$

at $\bar{n} = 0.5$. Hence the magnitude of the error incurred in the approximation depends upon the relative magnitude of the last three terms in the above equation. The magnitude of these terms can be checked readily by calculating from the initial approximate constants, taken from the formation curve, the relative concentrations in mole per cent. of each ionic species as a function of ammine concentration. Typical results of such calculations are given in Table II. It is immediately apparent that the sum of the terms $3[MA_2] + 5[MA_3] + 7[MA_4]$ is not a negligible quantity; however Table II does indicate that the relative error in the approximate constant at 15° is identical to that at 35°. This means that the temperature coefficient of the approximated constants is the same as for the corrected constants and therefore will serve equally well in the calculation of ΔH. In contrast, the calculation of ΔS requires a precise value for ΔF. Consequently the corrected constants were obtained for all dissociation constants at 25°, and ΔF values calculated from these constants; then ΔH values calculated as above were used in evaluating ΔS at 25°.

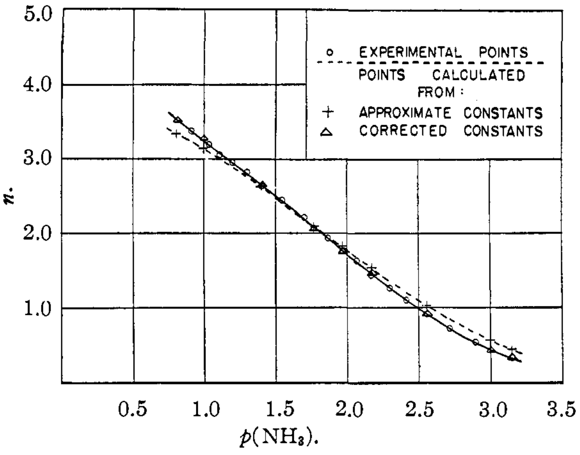

Fig. 1.—Comparison of experimental formation curve for the system cadmium(II)–ammonia with the theoretical curves calculated from the approximate and corrected dissociation constants.

TABLE II

RELATIVE CONCENTRATIONS IN MOLE PER CENT. OF THE VARIOUS IONIC SPECIES IN THE CADMIUM–METHYLAMINE SYSTEM AT VARIOUS VALUES OF $\bar{n}$[a] [CALCULATED FROM APPROXIMATE CONSTANTS]

			Solution composition, %				
$\bar{n}$	$-\log CH_3NH_2$	Temp., °C.	Cd^{++}	CdA^{++}	CdA_2^{++}	CdA_3^{++}	CdA_4^{++}
0.5	3.00	15	47.0	47.0	5.9	0.1	0.0
0.5	2.825	35	47.0	47.0	5.9	0.1	.0
1.0	2.510	15	18.5	57.4	22.8	1.3	.0
1.0	2.328	35	18.5	57.4	22.8	1.3	.0
1.5	2.109	15	5.6	43.7	43.7	6.8	.2
1.5	1.930	35	5.6	43.7	43.7	6.8	.2

[a] $\bar{n}$ = average number of amine molecules bound per metal ion.

The magnitude of the correction involved is seen in Table III where approximated and corrected constants for the cadmium ammines at 25° are compared.

TABLE III

	Approximate pk			Corrected pk		
	Cd^{++} CH_3NH_2	Cd^{++} NH_3	Cd^{++} en	Cd^{++} CH_3NH_2	Cd^{++} NH_3	Cd^{++} en
pk_1	2.905	2.940	5.92	2.745	2.741	5.84
pk_2	2.019	2.155	4.70	2.063	2.209	4.78
pk_3	1.214	1.506	2.07	1.131	1.372	2.07
pk_4	0.409	0.838		0.611	1.126	

Results

Figure 2 shows ln K plotted as a function of the reciprocal temperature for each of the systems investigated. In the case of ethylenediamine, K

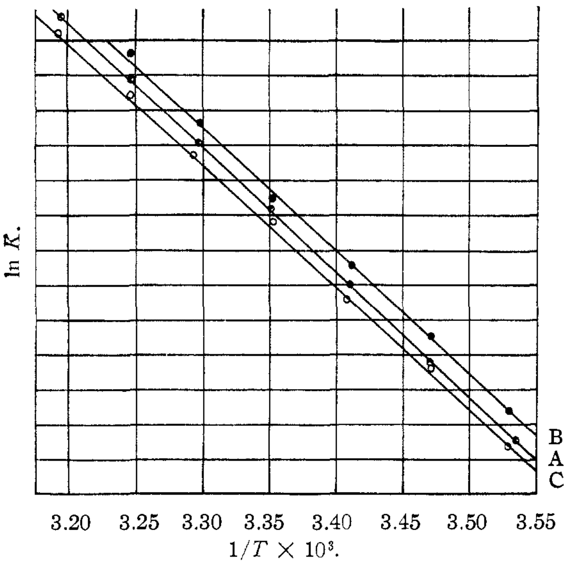

Fig. 2.—Temperature dependence of the dissociation constants of the complexes: $Cd(NH_3)_2^{++}$, A, -◐-◐-; $Cd(NH_2CH_3)_2^{++}$, B, -●-●-; $Cd(en)^{++}$, C, -○-○-. Each scale division of the ordinate = 0.1 ln K unit.

represents the first consecutive dissociation constant, or $K = k_1$. For the methylamine and ammonia complexes, K represents the product of the first two consecutive dissociation constants, or $K = k_1k_2$. Figure 3 shows a similar plot for the over-all dissociation constants of the ions $[Cden_2]^{++}$ and $[CdA_4]^{++}$ (*i.e.*, over-all constant = $pK_1 + pK_2$ for the ethylenediamine system and $pK_1 + pK_2 + pK_3 + pK_4$ for the ammonia and methylamine systems). The thermodynamic functions are summarized in Table IV.[9]

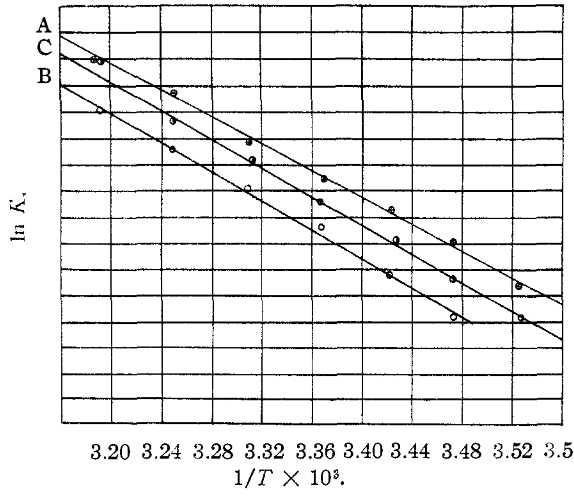

Fig. 3.—Temperature dependence of the dissociation constants of: $Cd(NH_3)_4^{++}$, A, -⊕-⊕-; $Cd(NH_2CH_3)_4^{++}$, B, -⊖-⊖-⊖-; $Cd(en)_2^{++}$, C, -◐-◐-◐-. Each scale division of the ordinate = 0.25 ln K unit.

The values given for the fully coördinated complexes are included for completeness although they are somewhat less precise than the values in the upper half of the table. The lower precision resulted primarily from the difficulty in measuring accurately small changes in pH in strongly alkaline solutions and from the nature of the dependence of the constant upon the pH. This was particularly true in the case of the methylamine complex for which the formation curve at $\bar{n} > 3.2$, (pH > 10), could be obtained only by extrapolation.

TABLE IV

SUMMARY OF THERMODYNAMIC FUNCTIONS AT 25°

Complex	pK (cor.)	ΔH, kcal./mole	ΔF, kcal./mole	$T\Delta S$, kcal./mole
$Cd(NH_3)_2^{++}$	4.950	7.12	6.75	0.37
$Cd(NH_2CH_3)_2^{++}$	4.808	7.02	6.56	.46
$Cd(en)^{++}$	5.836	7.03	7.96	− .93
$Cd(NH_3)_4^{++}$	7.44	12.7	10.16	2.53
$Cd(NH_2CH_3)_4^{++}$	6.55	13.7	8.94	4.77
$Cd(en)_2^{++}$	10.62	13.5	14.50	− .98

Discussion

Analysis of the data in Table IV shows that the standard free energy of dissociation of the $Cd(en)^{++}$ complex is 1.40 kcal. greater than that for the $Cd(NH_2CH_3)_2^{++}$ complex, indicating a considerably greater stability for the chelate compound. However, the heats of dissociation, $\Delta H°_{298}$, are 7.02 kcal. and 7.03 kcal., respectively. The uncertainty in the measurement of ΔH is approximately $\pm 1\%$, hence the two values are identical within the limits of the experimental data. In this instance, therefore, the increase in stability due to chelation can be attributed entirely to probability or entropy factors, the bond strength remaining unchanged as a result of chelation. It is also interesting to note that the less precise ΔH_{298} values for the complexes $Cd(en)_2^{++}$ and $Cd(NH_2CH_3)_4^{++}$ are identical within the limits of the measurement.

A closer analysis of the physical model for the probability factor indicates that one of the principal differences between the chelate and non-chelate structures lies in the fact that after the first metal–nitrogen bond has formed during complex formation, or after the first metal–nitrogen bond has broken during complex decomposition, the probability of forming the second metal–nitrogen bond is increased in the complex containing the chelating ligand. This results from the fact that the second nitrogen of the chelating ligand is held close to the metal cation, whereas a second non-chelating ligand is likely to be a greater distance away. If this is true, the difference in stability between chelate and non-chelate structures should diminish as the chain length of the chelating agent increases. This factor could then contribute appreciably to the stability of 5-member chelate rings and to the lower stability of bigger rings involving saturated ligands. The lower stability of 3- and 4-membered rings could result from steric strain which alters the bond strength in chelate structures enough to overshadow the entropy terms. According to this hypothesis, the maximum stability which is observed for a 5-membered chelate ring in complexes of the cadmium–ethylenediamine type results from a balance between the

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[9] In Fig. 3 data for the complex $Cd(NH_2CH_3)_4^{++}$ can be fitted to a curve somewhat better than to a straight line; however there is some question as to whether or not the precision of the data justifies a curve. In any event, the slope of the curve at 25° is the same as that of the straight line used in the figure.

competing ΔH and ΔS factors as the ring size is increased from three to higher members. A semi-quantitative treatment of this hypothesis is being subjected to experimental test.

It is also of interest to compare complexes of ammonia with the corresponding methylamine compounds. If their thermodynamic functions are comparable, the more abundant data for ammonia complexes can be used to compare entropy effects in cases where the data on the complexes of methylamine are unobtainable. Although Frank and Evans,[10] on the basis of unpublished work of Calvin, reported large differences between the thermodynamic functions of $Ni(NH_3)_6^{++}$ and $Ni(NH_2CH_3)_6^{++}$, the data for $Cd(NH_3)_2^{++}$ and $Cd(NH_2CH_3)_2^{++}$ shown in Table IV reveal almost negligible differences in the thermodynamic functions of the two cadmium complexes.

	ΔH, kcal./mole	ΔS, cal./deg. × mole
$Cd(NH_3)_2^{++}$	7.12	1.2
$Cd(NH_2CH_3)_2^{++}$	7.02	1.5
$Cd(NH_3)_4^{++}$	12.7	8.5
$Cd(NH_2CH_3)_4^{++}$	13.7	16.0

Although data for the complexes containing 4 ligands are much less precise than the data for ions containing 2 ligands, it is interesting to note that differences between the tetra-coördinated species (*i.e.*, $Cd(NH_3)_4^{++}$ as compared to $Cd(NH_2CH_3)_4^{++}$) are perhaps significant. The data of Basolo and Merman[11] for the copper(II) complexes of ethylenediamine and N-methylethylenediamine indicate a similar phenomenon. The placing of the methyl group on the nitrogen of the ethylenediamine may be considered analogous to the placing of the methyl group on the nitrogen of the ammonia. Their data, summarized below, indicate that the ΔH values for the $Cu(en)^{++}$ complex and the $Cu(N\text{-}Me\text{-}en)^{++}$ system are essentially the same, whereas ΔH values for bis complexes, $Cu(en)_2^{++}$ and $Cu(N\text{-}Me\text{-}en)_2^{++}$ differ somewhat.

	$\Delta H(0°)$	$\Delta S(0°)$[11]
$[Cu\ en]^{++}$	8.6	−21
$[Cu(N\text{-}Me\text{-}en)]^{++}$	8.5	−20
$[Cu(en)_2]^{++}$	17.2	−35
$[Cu(N\text{-}Me\text{-}en)_2]^{++}$	15.5	−36

Such a trend might be of importance in comparing the $Ni(NH_3)_6^{+6}$ and $Ni(NH_2Me)_6^{++}$ data of Calvin with data of other investigations.

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[10] H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 531 (1945).

[11] F. Basolo and R. Kent Murmann, THIS JOURNAL, **74**, 5243 (1952).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MALAYA]

The Roles of the Amino and Hydroxyl Groups of Collagen in its Reactions with Formaldehyde, Tannic Acid and Quinone

BY R. W. GREEN

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The controlled partial acetylation of collagen at its amino and hydroxyl radicals has been used to investigate the functions of these groups in some reactions with common tanning agents. The binding of formaldehyde, tannic acid and quinone is examined as a function of the degree of acetylation, and possible interpretations of the experimental data are discussed.

Introduction

The reactions of proteins with formaldehyde have given rise to an extensive literature which has recently been reviewed by French and Edsall[1] and by Gustavson.[2] Although a wide variety of protein groups can be involved, evidence from titration curves,[3] deamination,[4] acetylation[5] and from experiments with model substances[6] indicates that in neutral or weakly alkaline solution the most important reaction occurs at the ε-amino groups of lysine residues. The marked change in physical properties accompanying the binding of formaldehyde near *p*H 8 has led to the general opinion that covalent cross-linkages are introduced into the molecule in the form of methylene bridges.

In the particular case of collagen, the importance of free amino groups in formaldehyde tanning has been inferred from experiments[4,7] on deamination with nitrous acid, a reagent which is well known[8] to cause more than one side reaction. Controlled acetylation of specific side-chain groups[9] offers a less equivocal means of modifying the formaldehyde reaction and we describe here experiments with a range of collagen derivatives containing varying proportions of N- and O-acetyl groups. The effect of acetylation on the fixation of two other tanning agents, tannic acid and quinone, whose action is believed to be associated with amino groups[7,2] is also described.

Experimental

Acetylation.—Collagen, in the form of the standard hide powder of the International Society of Leather Trades' Chemists, was oven-dried and acetylated with anhydrous mixtures of acetic anhydride and acetic acid, as described by Green, Ang and Lam.[9] The derivatives, after thorough extraction with acetone, were exposed to the air of the laboratory and then again dried *in vacuo* at 100° over freshly-

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[1] D. French and J. T. Edsall, *Advances in Protein Chemistry*, **2**, 277 (1945).

[2] K. H. Gustavson, *ibid.*, **5**, 353 (1949).

[3] R. A. Kekwick and R. K. Cannan, *Biochem. J.*, **30**, 235 (1936).

[4] J. H. Highberger and E. W. Retzsch, *J. Am. Leather Chem. Assoc.*, **34**, 131 (1939).

[5] H. Nitschmann and H. Lauener, *Helv. Chim. Acta*, **29**, 184 (1946).

[6] H. Fraenkel-Conrat and H. S. Olcott, THIS JOURNAL, **70**, 2673 (1948).

[7] J. H. Bowes and R. H. Kenten, *Biochem. J.*, **44**, 142 (1949).

[8] H. S. Olcott and H. Fraenkel-Conrat, *Chem. Revs.*, **41**, 151 (1947).

[9] R. W. Green, K. P. Ang and L. C. Lam, *Biochem. J.*, in press.