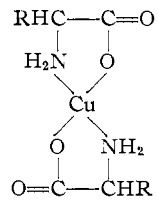


and (b) an absorption maximum around 700 mμ indicates a 1:1 complex. Our conclusion may be compared to that of Klotz, *et al.*,[5] who state that the absorption peak of copper(II) ion in complexes shifts toward shorter wave lengths, from 700 to 600 mμ, as coördination number is increased.

Figure 6, curves 1, 2 and 3 are similar to Fig. 5, except that here glycylglycinate is the ligand. Here all three curves have the same peak at 630 mμ. Therefore, from what has been said above, only one complex is present, namely, CuA_2.

It must be noted that in Figs. 4, 5, and 6 the *p*H varies from mixture to mixture, depending on the ratio of potassium serinate or potassium glycylglycinate to copper(II) nitrate in the mixture. This variation, however, has no effect on the conclusions obtained on the formulas of the complexes. It is seen in Table IV that when an alkali is added to a given copper–serine mixture for the purpose of varying the *p*H, the values of λ_{max} fall in the region 635 to 617 mμ over a *p*H range of 5.15 to 10.50. This wave length region of absorption maxima is, as noted above, an indication of the presence of a 1:2 complex. In addition it is noted in Table IV that over a *p*H range of 6.46 to 10.50 for the particular copper–serine mixture, the value of the molar extinction coefficient at the wave length of maximum absorption is almost constant. For these reasons we are confident that the spectrophotometric results are valid in spite of the variation in *p*H.

Summary on Number and Formulas of Complexes.—Tables I, II, III, and Fig. 4 show that the polarographic, potentiometric, conductometric and spectrophotometric methods are in agreement with respect to the existence of the 1:2 complex. The conductometric and spectrophotometric methods reveal an additional 1:1 complex whenever the ligand is an α-amino acid ion, but fail to show this when the ligand is the anion of glycylglycine.

PITTSBURGH 19, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY]

Stability of Zinc Complexes with Glutathione and Oxidized Glutathione[1]

BY NORMAN C. LI, OSCAR GAWRON AND GLORIA BASCUAS

RECEIVED JULY 24, 1953

Several acid dissociation constants of glutathione and oxidized glutathione and the stability of zinc complexes with glutathione and with oxidized glutathione were determined at 25°. Equations for calculation of the several acid dissociation constants by the Bjerrum method have been deduced and equilibrium formation constants for the 1:1 complexes, *i.e.*, 1 mole of zinc ion to 1 mole of chelating agent, are reported. It is suggested that in the zinc–glutathione complex, zinc is probably coördinated through the sulfur atom and the amino group, and that in the zinc–oxidized glutathione complex, the coördination is probably through the amino and α-carboxylate groups.

Because of current biochemical interest in glutathione and oxidized glutathione, this paper reports the determination of the formation constants of zinc complexes with glutathione and oxidized glutathione, by an adaptation of Bjerrum's method.[2] This method, which involves measurement of the *p*H of solutions containing known amounts of the metal ion, the peptide and a base, was carried out at a constant ionic strength of 0.15. This value of the ionic strength was chosen so as to be the same as the ionic strength used in zinc–albumin studies, inasmuch as the purpose of this study is to furnish background information on metal–protein complexes.

In order to obtain the concentration of the ligand, taken to be the anion of the peptide, it is necessary to determine the acid dissociation constants of the peptides. Values for pK_2 (COOH), 3.53, pK_3 (NH_3^+), 8.66, and pK_4(SH), 9.12, of glutathione have been listed by Cohn and Edsall.[3] Their pK_4 however, is in serious disagreement with the value, 9.62, reported by Pirie and Pinhey.[4] Because of this large discrepancy we have redetermined these values. The pK's of oxidized glutathione have not been reported.

Calculation of Constants

(A) Dissociation Constants of Oxidized Glutathione and Glutathione.—The dissociation constants for oxidized glutathione which are necessary for calculating formation constants are determined by the following equilibria present in aqueous solutions

$$H_4A = H_3A^- + H^+;\ K_3 = (H_3A^-)(H^+)/(H_4A) \quad (1)$$

$$H_3A^- = H_2A^{-2} + H^+;\ K_4 = (H_2A^{-2})(H^+)/(H_3A^-) \quad (2)$$

$$H_2A^{-2} = HA^{-3} + H^+;\ K_5 = (HA^{-3})(H^+)/(H_2A^{-2}) \quad (3)$$

$$HA^{-3} = A^{-4} + H^+;\ K_6 = (A^{-4})(H^+)/(HA^{-3}) \quad (4)$$

where ( ) represent molar concentration and H_4A is

$$\begin{array}{l} {}^{-}OOC\overset{b}{—}(NH_3^+)—CH—(CH_2)_2—CONH—CH—(CH_2S)—CONH—CH_2COO\overset{a}{H} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad | \\ {}^{-}OOC\overset{b}{—}(NH_3^+)—CH—(CH_2)_2—CONH—CH—(CH_2S)—CONH—CH_2COO\overset{a}{H} \end{array} \quad \text{I}$$

Removal of protons in the successive ionizations are assumed to take place in the order a,a,b,b for equations 1, 2, 3, 4, respectively.

(1) With the support of Grant No. 1496 from the Penrose Fund of the American Philosophical Society.

(2) J. Bjerrum, "Metal Ammine Formation in Aqueous Solutions," P. Haas & Sons, Copenhagen, 1941.

(3) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 85.

(4) N. W. Pirie and K. G. Pinhey, *J. Biol. Chem.*, **84**, 321 (1929).

Equations relating concentration of peptide, concentration of KOH and pH to the dissociation constants can be derived as follows: for oxidized glutathione, let T = total concn. of peptide in the solution

$$T = A^{-4} + HA^{-3} + H_2A^{-2} + H_3A^{-1} + H_4A \quad (5)$$

(XH) = total concn. of protons bound to peptide in the solution

$$(XH) = HA^{-3} + 2H_2A^{-2} + 3H_3A^{-1} + 4H_4A \quad (6)$$

$$(XH) = 4T - (KOH) - (H^+) + (OH^-) \quad (7)$$

$$n = (XH)/T \quad (8)$$

Combinations of equations 1, 2, 3, 4, 5, 6 and 8 gives

$$n = \frac{K_3K_4K_5(H^+) + 2K_3K_4(H^+)^2 + 3K_3(H^+)^3 + 4(H^+)^4}{K_3K_4K_5K_6 + K_3K_4K_5(H^+) + K_3K_4(H^+)^2 + K_3(H^+)^3 + (H^+)^4} \quad (9)$$

In deriving equations for K_3 and K_4, we may neglect the terms in eq. 9 which contain K_5, K_6, since $K_5, K_6 <<< K_3, K_4$. The following equations then result

$$pK_3 = pH + \log \frac{n-3}{4-n} + \log\left[1 + \frac{K_4(n-2)}{(H^+)(n-3)}\right] \quad (10)$$

and

$$pK_4 = pH + \log \frac{n-2}{3-n} - \log\left[1 + \frac{(H^+)(4-n)}{K_3(3-n)}\right] \quad (11)$$

In deriving equations for K_5 and K_6, where the solution is basic, the last two terms on the right-hand side of equations 5 and 6 may be dropped, so that

$$n = \frac{K_5(H^+) + 2(H^+)^2}{K_5K_6 + K_5(H^+) + (H^+)^2}$$

The following equations then result

$$pK_5 = pH + \log \frac{n-1}{2-n} + \log\left[1 + \frac{K_6n}{(H^+)(n-1)}\right] \quad (12)$$

$$pK_6 = pH + \log \frac{n}{1-n} - \log\left[1 + \frac{(H^+)(2-n)}{K_5(1-n)}\right] \quad (13)$$

Similar equations for the constants of glutathione can be derived. The following are the simplified equations

$$pK_2(COOH) = pH + \log \frac{n-2}{3-n} \quad (14)$$

$$pK_3(NH_3^+) = pH + \log \frac{n-1}{2-n} + \log\left[1 + \frac{K_4n}{(H^+)(n-1)}\right] \quad (15)$$

$$pK_4(SH) = pH + \log \frac{n}{1-n} - \log\left[1 + \frac{(H^+)(2-n)}{K_3(1-n)}\right] \quad (16)$$

Equation 7 for glutathione becomes

$$(XH) = 3T - (KOH) - (H^+) + (OH^-)$$

where T is the total concentration of glutathione in the solution. The formation function n is still given by equation 8.

(B) Formation Constants of Metal–Peptide Complexes.—The chelating agent is taken as the species from which all ionizable protons have been removed. In the case of glutathione, this assumption is based on an analogy with cysteine which has been shown by Albert[5] to have all ionizable protons removed when it functions as a chelating agent with a metal ion. In the case of oxidized glutathione, see Structure I, this assumption is justified by analogy with the work of Li and Doody,[6] who show that little chelation takes place whenever an α-NH_3^+ group is present.

The following symbols will be used

T_M = total concn. of metal ion in soln.

T = total concn. of peptide in soln.

A = total concn. of free chelating agent in soln. From what has been said, the chelating agents are A^{-3} and A^{-4} when the peptides are glutathione and oxidized glutathione, respectively.

$\bar{n}$ = av. no. of moles of ligand bound per mole of metal ion

$$\bar{n} = (MA + 2MA_2)/T_M \quad (17)$$

For oxidized glutathione

$$T_M = M^{+2} + MA^{-2} + MA_2^{-6} \quad (18)$$

$$T = A^{-4} + HA^{-3} + H_2A^{-2} + MA^{-2} + 2MA_2^{-6} + H_3A^{-1} + H_4A \quad (19)$$

At the pH used, the last two terms in equation 19 are negligible. Introducing equation 17, equation 19 now becomes

$$T = A^{-4} + HA^{-3} + H_2A^{-2} + \bar{n}T_M$$

Combining this with equations 3 and 4, we obtain

$$\bar{n} = \frac{T - A(K_5K_6 + K_5(H^+) + (H^+)^2)/K_5K_6}{T_M} \quad (20)$$

From equations 3, 4, 6, 7 and again neglecting H_3A^{-1} and H_4A, we obtain the equation

$$A^{-4} = \frac{SK_5K_6}{K_5(H^+) + 2(H^+)^2} \quad (21)$$

where

$$S = 4T - (KOH) - (H^+) + (OH^-)$$

It can readily be shown that equations 20 and 21 become, in the case of glutathione

$$\bar{n} = \frac{T - A(K_3K_4 + K_3(H^+) + (H^+)^2)/K_3K_4}{T_M} \quad (22)$$

and

$$A^{-3} = \frac{S'K_3K_4}{K_3(H^+) + 2(H^+)^2} \quad (23)$$

where

$$S' = 3T - (KOH) - (H^+) + (OH^-)$$

These values of n and pA, the negative logarithm of A, when substituted into the following equations, yield the values of the formation constants of the MA (1:1) and MA_2 (1:2) complexes.

$$\log k_1 = pA + \log \bar{n}/(1 - \bar{n}) \quad (24)$$

$$\log k_2 = pA + \log (\bar{n} - 1)/(2 - n) \quad (25)$$

It is seen from equations 24 and 25 that at $n = 0.5$ and at $n = 1.5$, pA equals to log k_1 and log k_2, respectively.

Experimental

Glutathione in the reduced state was a C.P. Pfanstiehl product. Stock solutions about 0.01 M in glutathione were prepared in air-free water and standardized against carbonate-free potassium hydroxide using a pH meter. Only freshly prepared stock solutions were used. Oxidized glutathione was a Schwarz product and contained about 14% associated alcohol. The material was dried *in vacuo* at 56° to constant weight. Preparation and analyses of stock solutions (0.01 M) were done in the same way as for gluta-

(5) A. Albert, *Biochem. J.*, **50**, 693 (1952).

(6) N. C. Li and E. Doody, THIS JOURNAL, **74**, 4184 (1952).

thione. All the other chemicals were commercial C.P. reagent grade products.

The peptides in 0.15 M aqueous potassium nitrate solutions were titrated at 25° with standard potassium hydroxide solutions both in the absence and presence of metal ion. Nitrogen was bubbled through the solution throughout the titrations. pH measurements were made with a Beckman Model G pH meter. Buffer solutions, pH's of 4 and 7, were used to standardize the instrument.

Conductometric titration was done in an apparatus previously described.[7]

Results

Table I shows the values obtained for pK_2, pK_3 and pK_4 of glutathione. The simplified equations 14, 15 and 16 were used for calculations of these constants. Calculations of pK_2 are independent of pK_3 and pK_4 because pK_2 differs from pK_3 and pK_4 by more than 5 pK units. Since pK_3 and pK_4 differ from each other by less than 1 pK unit, equations used to calculate either one must involve the value of the other.

TABLE I

DISSOCIATION CONSTANTS OF GLUTATHIONE AT 25°

(15.00 ml. solution containing 0.006388 M glutathione and 0.15 M KNO_3, plus x ml. 0.0390 N KOH).

A. Determination of pK_2

x, ml.	pH	n	$\log (n-2)/(3-n)$	pK_2
0.30	3.20	2.778	0.54	3.74
0.60	3.32	2.680	.33	3.65
0.90	3.48	2.578	.14	3.62
1.10	3.58	2.509	.02	3.60
1.30	3.70	2.439	−0.11	3.59
1.60	3.88	2.328	− .31	3.57
1.90	4.12	2.221	− .55	3.57
			Av.	3.59

B. Determination of pK_3

$$a = \log\left[1 + \frac{K_4 n}{(H^+)(n-1)}\right]$$

x, ml.	pH	n	$\log (n-1)/(2-n)$	a	pK_3
2.50	7.02	1.982	1.74	0.00	8.76
2.60	7.52	1.942	1.21	.01	8.74
2.80	7.94	1.860	0.79	.02	8.75
3.30	8.42	1.657	.28	.06	8.76
3.60	8.60	1.535	.06	.09	8.75
3.80	8.70	1.453	−.08	.13	8.75
				Av.	8.75 ± 0.005

C. Determination of pK_4

$$b = -\log\left[1 + \frac{(H^+)(2-n)}{K_3(1-n)}\right]$$

x, ml.	pH	n	$\log n/(n-1)$	b	pK_4
5.40	9.40	0.802	0.61	−0.37	9.64
5.80	9.60	.639	.25	− .19	9.66
6.10	9.74	.517	.03	− .12	9.65
6.30	9.82	.451	−0.09	− .09	9.64
				Av.	9.65 ± 0.008

Figure 1 shows the results obtained for oxidized glutathione. From equations 10 and 11, approximate values of pK_3 and pK_4 are given by the pH's at $n = 3.5$ and $n = 2.5$, respectively. From Fig. 1, it is seen that these values are: $pK_3 = 3.15$ and $pK_4 = 4.03$. Since the ligand for the zinc–peptide complex is A^{-4} and since $pK_{4(COOH)}$ differs from $pK_{5(NH_3^+)}$ by more than 4.5 pK units, we have not proceeded to determine the final values of pK_3 and pK_4.

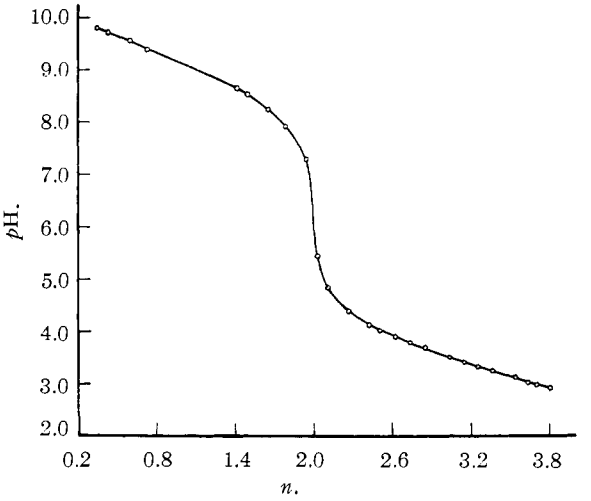


Fig. 1.—n vs. pH for oxidized glutathione at 25.0°.

The values of pK_5 and pK_6 of oxidized glutathione are involved in the calculation of the formation constants of the zinc complex. These pK's were determined in the same way as were the pK_3 and pK_4 of glutathione, and the results are summarized in Table II.

TABLE II

DISSOCIATION CONSTANTS OF OXIDIZED GLUTATHIONE AT 25°

$$a = \log\left[1 + \frac{K_6 \quad n}{(H^+)(n-1)}\right]$$

$$b = -\log\left[1 + \frac{(H^+)(2-n)}{K_5(1-n)}\right]$$

pH	n	$\log (n-1)/(2-n)$	a	pK_5
7.28	1.95	1.28	0.00	8.56
7.90	1.79	0.58	.02	8.50
8.24	1.66	.29	.05	8.58
8.52	1.50	.00	.10	8.62
8.64	1.42	− .14	.14	8.64
			Av.	8.57 ± 0.04

pH	n	$\log (n/(1-n)$	b	pK_6
9.38	0.729	0.43	−0.25	9.56
9.56	0.599	.17	− .14	9.59
9.72	.431	− .12	− .08	9.52
9.80	.352	− .26	− .06	9.48
			Av.	9.54 ± 0.04

From the data in Tables I and II it is seen that $pK_{5(NH_3^+)}$, 8.57, and $pK_{6(NH_3^+)}$, 9.54, of oxidized glutathione are smaller and larger, respectively, than the $pK_{3(NH_3^+)}$, 8.75, of glutathione, and that pK_6 is smaller than $pK_{4(SH)}$, 9.65, of glutathione. This situation is the same as the analogous cysteine–cystine couple, in which the $pK_{NH_3^+}$ of cysteine, 8.18, is in between the two $pK_{NH_3^+}$'s of cystine, 7.85 and 9.85.[3]

With the use of the pK_3 and pK_4 of glutathione, and pK_5 and pK_6 of oxidized glutathione, the quantities $\bar{n}$ and pA were calculated from equations 20, 21, 22 and 23. Tables III and IV contain data on zinc–peptide complexes. Logarithms of the first formation constants, k_1, are calculated from equa-

(7) N. C. Li and E. Doody, THIS JOURNAL, 72, 1891 (1950).

tion 24, and are placed in the last column of the two tables. From equation 24 it is seen that the most probable log k_1 is the value of pA at $n = 0.5$.

TABLE III

FORMATION CONSTANTS OF ZINC–GLUTATHIONE COMPLEX

(15.00 ml. solution containing 0.006388 M glutathione, 0.15 M KNO_3 and 0.003303 M zinc nitrate, plus x ml. 0.0797 N KOH)

x, ml.	pH	$\bar{n}$	pA	$\log \bar{n}/(1-\bar{n})$	log k_1
1.60	5.92	0.317	8.88	−0.33	8.55
1.80	6.20	.482	8.37	− .03	8.34
2.00	6.42	.639	7.99	+0.25	8.24
2.20	6.72	.806	7.45	.62	8.07
2.40	7.08	.954	6.80	1.32	8.12

log k_1 (most probable value, see text) = 8.30

x, ml.	pH	$\bar{n}$	pA	$\log (n-1)/(2-\bar{n})$	Sum of col. 4 and 5, eq. 25
2.60	7.50	1.104	6.06	−0.93	5.13
2.80	8.02	1.228	5.14	− .53	4.61
3.00	8.68	1.267	4.07	− .43	3.64
3.20	9.18	1.282	3.46	− .41	3.05
3.40	9.58	1.401	3.21	− .17	3.04

TABLE IV

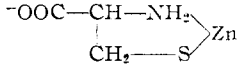
FORMATION CONSTANT OF ZINC-OXIDIZED GLUTATHIONE COMPLEX AT 25°

(15.00 ml. solution containing 0.006346 M oxidized glutathione, 0.15 M KNO_3 and 0.003303 M zinc nitrate, plus x ml. 0.0797 N KOH)

x, ml.	pH	$\bar{n}$	pA	$\log \bar{n}/(1-\bar{n})$	log k_1
2.60	6.06	0.167	8.30	−0.70	7.60
2.80	6.36	.330	7.75	− .31	7.44
3.00	6.60	.479	7.32	− .04	7.28
3.20	6.84	.638	6.90	+ .25	7.15
3.40	7.12	.798	6.40	.60	7.00
3.60	7.56	.927	5.61	1.10	6.71

log k_1 (most probable value, see text) = 7.22

From Table III it is seen that log k_1 is 8.30 for the zinc–glutathione complex. If the chelation of 1 mole of zinc to 1 mole of glutathione were through the α-amino and α-carboxylate groups, the value of log k_1 should be much smaller. Thus the values of log k_1[8] of zinc glycinate and zinc alaninate complexes, wherein chelation is through the α-amino and α-carboxylate groups, are only 5.52 and 5.21, respectively. Albert[5] deduced that the structure of the zinc chelate of cysteine is



and gives a log k_s of 18.2, where k_s is k_1k_2. In the case of zinc complexes with glycinate and alaninate,[8] log k_1 is about 1 unit higher than log k_2, so that a reasonable value for log k_1 for the zinc–cysteine complex may be of the order of 9.5. In a zinc chelate where the bonds are Zn-S and Zn–NH_2 but where the chelate ring is not 5-membered as in zinc–cysteine, the log k_1 would be expected to be smaller than 9.5. Our log k_1, 8.30, for the zinc–glutathione complex, therefore, indicates that zinc is probably coördinated through the sulfur atom and the amino group, rather than through the amino and carboxyl groups.

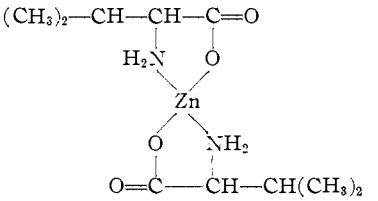
In Table III it is also seen that for $\bar{n}$ higher than 1, the values of pA and $(p\text{A} + \log (\bar{n}-1)/(2-\bar{n}))$ decreases very rapidly up to $\bar{n}$ of about 1.3 and that the highest $\bar{n}$ is 1.4. For these reasons log k_2 is not calculated.

The value of log k_1 for the zinc–oxidized glutathione complex is given in Table IV as 7.22 and the maximum $\bar{n}$ about 0.93. The presence of only one complex of the MA type, *i.e.*, in which the mole ratio of zinc ion to oxidized glutathione anion is 1:1 is confirmed by potentiometric and conductometric titrations of zinc ion with oxidized glutathione anion, when the latter is in its most basic form. These titrations were performed in a manner previously described by Li and Doody.[6,7] The further formation of a 2:1 chelate would necessitate the following reaction

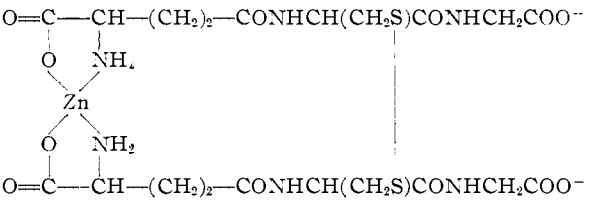
$$ZnA^{-2} + A^{-4} = ZnA_2^{-6}$$

Electrical repulsion between the 1:1 chelate ion and the most basic anion of oxidized glutathione, A^{-4}, would greatly inhibit the reaction.

Our value of log k_1 for this chelate is smaller than the values of log k_1k_2[9] for the zinc chelates of glycine, 9.96, alanine, 9.54, leucine, 8.93, and valine, 8.10. Since the 2:1 chelate of valine is probably



we propose as a likely structure for the peptide complex



The lower value of log k_1k_2 for the zinc–valine complex as compared to the log k_1k_2 for the zinc–glycine complex may be attributed to interference of the bulky isopropyl group of valine with formation of a chelating bond between the valine amino group and the metallic cation. An explanation of the lower value of log k_1 for the zinc-peptide complex as compared to log k_1k_2 for the zinc–valine complex would have to await confirmation of the structure of the zinc–peptide complex.

It must be mentioned that in our calculations we have used the assumption that (H^+) equals approximately to $10^{-p\text{H}}$. At an ionic strength of about 0.15, this may produce an error of 0.1 in the log k_1 of the zinc complexes with glutathione and oxidized glutathione. In view of the large uncertainties involved in the exact conversion of pH to (H^+), however, we have made the simplifying

(8) C. B. Monk, *Trans. Faraday Soc.*, **47**, 297 (1951).

(9) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelates," Prentice–Hall, Inc., New York, N. Y., 1952, pp. 526–531.

assumption inasmuch as the small corrections obtained with the use of activity coefficients would not affect our discussion on the probable structures of the chelates.

Preliminary experiments have been done on copper(II)–oxidized glutathione complex. The indication is that again only one complex of the MA type is formed and that the copper chelate is much more stable than the corresponding zinc chelate. These are as expected. More work on the copper-(II) chelate is being continued at this Laboratory.

Acknowledgment.—We wish to thank the American Philosophical Society for a grant which enabled us to carry out this investigation.

PITTSBURGH, PA.

[CONTRIBUTION NO. 123 FROM THE UNIVERSITY OF TENNESSEE, DEPARTMENT OF CHEMISTRY]

Platinum Oxide Catalysts

BY C. W. KEENAN, BETTY W. GIESEMANN AND HILTON A. SMITH

RECEIVED AUGUST 17, 1953

The sodium present in Adams platinum catalyst fusion product has been shown to be associated with a strong proton acceptor. The sodium can be largely removed by washing with acid, even quite dilute acid. The sodium found in the fusion mass is probably a reaction product whose precursor is sodium oxide formed by the thermal decomposition of the sodium nitrate melt. This study indicates that in the case of the hydrogenation of benzene, in the presence of Adams catalyst the need for additives or solvents is due to the effect such substances have in reacting with or removing the sodium component from the catalyst. A platinum oxide catalyst free of sodium has been prepared. It will catalyze the hydrogenation of benzene in the absence of additives or solvents.

Introduction

Experimental studies of the hydrogenation of benzene at room temperature and hydrogen pressures up to three or four atmospheres have indicated that this compound is not hydrogenated in the presence of Adams platinum catalyst alone. The use of methanol as a solvent is ineffective; however, when acids such as acetic acid are employed as solvent, the benzene readily accepts hydrogen.[1,2] It has been found in this Laboratory that certain organic additives of the quaternary ammonium salt type will promote the hydrogenation of benzene in the presence of platinum.[3] Moreover, if Adams platinum oxide is prereduced in acetic acid or methanol and subsequently thoroughly rinsed, the catalyst thus prepared will enable the hydrogenation of benzene either alone or in methanol.[4]

A comparison of the rates of hydrogenation and of deuteration of benzene cannot be made in the presence of a solvent such as acetic acid since the acidic proton of the solvent undergoes rapid exchange with gaseous deuterium in the presence of platinum.[5] Therefore it was of interest both to find out what prevented the hydrogenation of benzene in the absence of additives or pre-treatment of the catalyst, and also to prepare a platinum catalyst which by itself would be effective in causing the hydrogenation of benzene at ordinary temperatures and pressures.

Experimental

Adams Platinum Oxide.—Unless otherwise noted the fusion products were prepared according to the standard procedure[6] except that the temperature cycles followed were based on those described in an earlier paper.[7] For comparison, preparations of fusion products were also made according to a recent modification of the Adams procedure.[8]

Platinic Acid.—Platinic acid was prepared according to the directions of Wohler.[9,10] The several preparations were purified until they gave negative or questionable flame tests for sodium.

Anal. Calcd. for $H_2Pt(OH)_6$: Pt, 65.2; H_2O, 24.1. Found: Pt, 64.5; H_2O, 24.3.

Partially Dehydrated Platinic Acid.—A material with the approximate composition of the monohydrate of platinic oxide was prepared by the careful dehydration of platinic acid at 95° for one week.

Anal. Calcd. for $PtO_2 \cdot H_2O$: Pt, 79.6; H_2O, 7.3. Found: Pt, 77.0; H_2O, 9.6.

Sodium Nitrate.—The sodium nitrate was J. T. Baker C.P. Samples of this substance were heated for 10-minute intervals and then tested for basicity. Temperatures up to about 450° did not result in enough decomposition to cause distilled water solutions of the salt to be basic to litmus or phenolphthalein. However, the aqueous solutions of fusions above 500° appeared strongly basic. Duplicate fusions at 600° of 5-g. samples of sodium nitrate resulted in a basicity of 0.0052 meq. per gram. At 700° the basicity found was 0.233 meq. per gram.

Chloroplatinic Acid.—The chloroplatinic acid was obtained from the American Platinum Company.

Acetic Acid, Methanol, Benzene.—Commercial samples of high quality were fractionated at atmospheric pressure as needed in a 6-foot column packed with glass helices (equivalent to approximately 30 theoretical plates). Middle fractions of constant boiling points were collected for use.

Hydrogen Gas.—The gas was obtained from the National Cylinder Gas Company.

Analyses.—Platinum was determined by the electrolytic precipitation of the metal on a tared platinum electrode.[11] All samples were first dissolved in aqua regia. It was found that the sodium nitrate–chloroplatinic acid fusion

(1) R. Adams and J. R. Marshall, THIS JOURNAL, **50**, 1970 (1928).

(2) H. A. Smith, D. M. Alderman and F. W. Nadig, *ibid.*, **67**, 272 (1945).

(3) H. A. Smith and W. H. King, unpublished work.

(4) J. Young and H. A. Smith, unpublished work.

(5) Lloyd E. Line, Jr., Betty Wyatt and Hilton A. Smith, THIS JOURNAL, **74**, 1808 (1952).

(6) R. Adams, V. Voorhees and R. L. Shriner, "Organic Syntheses," Col. Vol. I, John Wiley and Sons, Inc., New York, N. Y. 1932, p. 452.

(7) R. Adams and R. L. Shriner, THIS JOURNAL, **45**, 2171 (1923).

(8) V. L. Frampton, J. D. Edwards, Jr., and H. R. Henze, *ibid.*, **73**, 4432 (1951).

(9) L. Wohler, *Z. anorg. Chem.*, **40**, 434 (1904).

(10) For the purpose of qualitative analysis an X-ray pattern of platinic acid was obtained. The data may be of interest to others since they have not been reported previously. X-Ray diffraction pattern of $H_2Pt(OH)_6$: 4.47 (s), 4.19 (s), 3.66 (m), 3.54 (m), 2.69 (m), 2.39 (m), 2.27 (m), 2.18 (m). There were over a dozen other weak lines visible.

(11) A. Schleicher, "Die Chemische Analyse," W. Bottger, Editor, "Electroanalytische Schnellmethoden," IV/V, Ferdinand Enke, Stuttgart, 1947, p. 86.