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Association Constants of Methylmercury with Sulfhydryl and Other Bases

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The distribution constants of methylmercuric halides between water and toluene were measured. The association constants of methylmercuric ion with various (Brönsted) bases, including those common in proteins, were measured and found to be in general much smaller than the association constants of mercuric ion with these bases. The greatest difference found was for the sulfhydryl group of cysteine or glutathione, which has an association constant of about $10^{15.8}$ for methylmercury and about $10^{20.3}$ for a mercury valence, according to Stricks and Kolthoff. Certain discrepancies suggested a recalculation of the equilibrium in the reaction of a bifunctional organic mercurial (XHgR'HgX) with the single sulfhydryl groups of two human serum mercaptalbumin (HSA) molecules to form a dimer (ASHgR'HgSA). If the affinity of cyanide for ASHgR'Hg^+ is taken equal to that of cyanide for CH_3Hg^+, instead of for mercury, a recalculation of the effect of cyanide on the dissociation of the dimer changes the rough estimate of the equilibrium constant (ASHgR'HgSA)/(ASHgR'Hg^+)(AS^-) from $10^{18.2}$ to about $10^{14.9}$. On the basis of the affinity of CH_3Hg^+ for Cl^- and Br^- the equilibrium constant for the mercury dimer of human mercaptalbumin is similarly revised from $10^{13.5}$ to about 10^{12}. These figures for albumin all involve an assumption of pK 10 for the sulfhydryl group. Independent of this assumption, the mercaptide association constant of CH_3HgSA is about 10^5 greater than that of ASHgSa, and 10^2 or 10^3 greater than that of ASHgR'HgSa.

Hughes[1a] pointed out the advantages of methylmercury as a reagent for protein sulfhydryl groups —its high specificity, mono-functionality and small size. He also determined its association constant with sulfhydryl, but included in the association constant was the distribution constant of methylmercuric iodide between toluene and water. He later[1b] published the absolute association constant but without accompanying experimental data. In this paper the distribution constants of the methylmercuric halides are measured, and the association constants of methylmercuric ion with various bases (in the Brönsted sense of proton acceptors) are determined. These data allow a more accurate calculation of the equilibrium constants for the dimerization of mercaptalbumin.

Experimental

A 17% aqueous methylmercuric hydroxide solution was a gift from Panogen, Inc., of Ringwood, Illinois.

Methylmercuric nitrate and chloride solutions were made by titration of methylmercuric hydroxide solution with the appropriate acid to a pH corresponding to that of a solution of the pure salt. Methylmercuric chloride solutions were also made from the solid (m.p. 160–168°), lit.[2] 167° which was recrystallized (from 15 times its weight of absolute ethanol) from samples containing about 15% insoluble impurity, one purchased from K & K Laboratories, Jamaica, New York, and one donated by Metalsalts Corporation, Hawthorne, New Jersey. Methylmercuric bromide (m.p. 160.8–161.2°) lit.[2] 161° was a gift from Dr. Walter L. Hughes. Methylmercuric iodide (m.p. 145–146°), lit.[3] 152°, was prepared by the method of Maynard.[3]

Solutions of a more strongly complexing anion were also made from CH_3HgOH or CH_3HgCl by adding that ion in equivalence or excess, since the reaction

$$CH_3HgCl + Br^- \longrightarrow CH_3HgBr + Cl^-$$

would occur practically quantitatively.

All other chemicals were reagent grade.

All experiments were run at 24–25°.

A Beckman Model G pH meter, a Beckman model DU spectrophotometer and a Sargent Model XXI polarograph were used. For measurement of the half wave potential an auxiliary potentiometer or standard resistor was attached to the polarograph. The potentials were measured with respect to a saturated calomel electrode. Polarographically methylmercury behaves similarly to other organo mercurials[4] showing two waves of equal height. The height and half-wave potential of the first wave only were used in the calculations. 0.01% gelatin was found to be more effective than methyl red as a maximum suppressor and was therefore used.

The method for the dithizone titration of methylmercury has already been described.[5]

Methods and Results

The equilibrium constant

$$K_3^{RS} = \frac{[CH_3HgSR]}{[CH_3Hg^+][RS^-]} \qquad (1)$$

(where RS^- represents the ionized form of sulfhydryl compound) may be determined as the product of several constants

$$K_3^{RS} = K_1^X D_X K_3^X K_{RSH} = K_2 K_{RSH} \qquad (2)$$

$$K_1^X = \frac{[CH_3HgSR][H^+][X^-]}{[CH_3HgX(\text{toluene})][RSH]} \qquad (3)$$

where CH_3HgX (toluene) represents the concentration of methylmercuric halide in a toluene layer that has been equilibrated with an aqueous layer containing the other species.

D_X represents the distribution constant [CH_3HgX (toluene)]/[CH_3HgX (aqueous)].

$$K_3^X = \frac{[CH_3HgX]}{[CH_3Hg^+][X^-]} \qquad (4)$$

is the association constant of the halide X^- with CH_3Hg^+.

$$K_{RSH} = \frac{[RSH]}{[H^+][RS^-]} \qquad (5)$$

is the association constant of the sulfhydryl group.

$$K_2 = \frac{[CH_3HgSR][H^+]}{[CH_3Hg^+][RSH]} \qquad (6)$$

Determination of K_1^X.—Several experiments were done to determine K_1^{Br} with cysteine (cys) and glutathione (GSH). Inasmuch as known[6]

(1) (a) W. L. Hughes, Jr., *Cold Spring Harbor Symposia*, XIV, 79 (1950); (b) W. L. Hughes, *Ann. N. Y. Acad. Sci.*, **65**, 454 (1957).

(2) N. V. Sidgwick, "The Chemical Elements and Their Compounds," Vol. I, Oxford Univ. Press, 1950, p. 310.

(3) J. L. Maynard, *J. Am. Chem. Soc.*, **54**, 2108 (1932).

(4) R. Benesch and R. E. Benesch, *ibid.*, **73**, 3391 (1951); *J. Phys. Chem.*, **56**, 648 (1952).

(5) R. B. Simpson and H. A. Saroff, *J. Am. Chem. Soc.*, **80**, 2129 (1958).

(6) The "known" amounts of sulfhydryl are on the basis of distribution experiments with an aliquot of the thiol solution and a few per cent. excess of CH_3HgBr at 0.0067 M bromide and pH 5.4, where essentially all the thiol should be combined with methylmercury. These showed that the SH content of the glutathione was about 1% and of the cysteine several per cent. below that calculated for the pure compound. Since the impurity probably was disulfide and therefore inert in the reaction with methylmercury, a recrystallization was not deemed necessary.

amounts of CH_3HgBr and thiol were added, the concentrations of CH_3HgSR and RSH (cysteine or glutathione) could be calculated from the methylmercury analysis of the toluene phase, since practically all of the methylmercury in the aqueous phase is combined with the thiol.

The concentrations at equilibrium are given in Table I.

TABLE I

CONCENTRATION ($M \times 10^4$) AT EQUILIBRIUM IN THE REACTION

$$CH_3HgBr + RSH = CH_3HgSR + H^+ + Br^-$$

CH_3HgBr (in toluene)	CH_3HgSR	RSH	Br^-	pH[a]	K_1^{Br}, M
2.0	3.7	0.9(cys)	3.7	2.14[1]	$10^{-1.26}$
2.9	2.8	1.8(cys)	2.8	1.53[1]	$10^{-1.35}$
2.8	3.0	1.7(cys)	53	2.71[2]	$10^{-1.19}$
1.7	4.0	0.67(cys)	54	3.52[3]	$10^{-1.24}$
3.8	1.2	3.7(GSH)	201	2.75[2]	$10^{-1.52}$
3.0	1.9	3.0(GSH)	102	2.75[2]	$10^{-1.42}$
2.5	2.4	2.5	52	2.75[2]	$10^{-1.45}$
2.1	2.8	2.1	28	2.75[2]	$10^{-1.50}$

[a] The pH was fixed by the use of 0.1 M buffers (1) sulfate-bisulfate, (2) chloroacetate, (3) acetate, and measured with the glass electrode.

Distribution Constants.—The distribution constants are given in Table II. They were determined by analyzing for methylmercury in the toluene layer by the dithizone titration and in the aqueous layer by polarographic wave heights.[7] In experiments with methylmercuric iodide at lower concentrations, CH_3HgI in the aqueous phase was determined by extraction from a large aliquot of the aqueous phase with new toluene and titration of the CH_3HgI in this toluene phase.

TABLE II

$$\text{Distribution constants } D_X = \frac{CH_3HgX\ (\text{toluene})}{CH_3HgX\ (\text{aqueous})}$$

$$D_{Cl} = \frac{5.2 \times 10^{-3}}{4.9 \times 10^{-4}} = 11$$

$$D_{Br} = \frac{2.2 \times 10^{-2}}{4.8 \times 10^{-4}} = 45$$

$$D_I = \frac{7.0 \times 10^{-2}}{2.3 \times 10^{-4}} = 300$$

$$D_I = \frac{5.8 \times 10^{-4}}{1.1 \times 10^{-6}} = 500$$

Corrections were made for the decrease in volume of the toluene phase calculated from the solubility of toluene in water (0.06 ml. per 100 ml.).

Only about 90% of the added methylmercuric halides could be accounted for by analysis. Since relative volumes of water and toluene were chosen so as to yield nearly equal amounts of methylmercury in the two phases, the amount unaccounted for was also assumed to be split equally between the two phases.

The above experiments were carried out in about 0.001 M added halide to suppress the ionization of the methylmercuric halide. In an experiment to determine the effect of ionic strength, the distribution constant of CH_3HgBr in 0.1 M KBr was found to be within the experimental error (about 10%) of the value in 0.001 KBr.

The distribution constant for CH_3HgI appeared to decrease with increasing concentration, but this decrease was not much greater than experimental error.

Methods of Determining K_3.—Values of the log of the association constant K_3 of methylmercury with various anions and bases are given in Table III. Methods of determining K_3 included:

TABLE III

LOG OF ASSOCIATION CONSTANT OF BASE IN FIRST COLUMN WITH CATION AT HEAD OF COLUMN[a]

	Hg^{++}, HgX^+ "average"	CH_3Hg^+	H^+
OH^-	10.85	9.5[b]	14
Cl^-	6.6	5.45[b,c]	<1
Br^-	8.4	6.7[b,c]	<1
I^-	11.9	8.7[b,c]	<1
SCN^-	8.7	6.1[c]	<1
CN^-	17.35	14.2[d]	9.3
Acetate		~3.6[e]	4.8
$HEDTA^{-3}$		6.2[d]	6.2
Phenolate		~6.5[e]	9.8
Pyridine	5.0	4.8[d]	5.3
NH_3	8.7	8.4[d]	9.3
Imidazole	8.35	7.3[d]	7.1
NH_2(his)	10.6	8.8[d]	9.1
Im(his)	7.5	6.4[e]	6.1
RS^-(cys)	20.1	15.7	8.6
RS^-(his)	20.5	15.9	9.0

[a] The values for mercury are logs of $\sqrt{[Hg\ X_2]/[Hg^{++}][X^-]^2}$. References and ionic strengths ($\Gamma/2$) for these are: OH^- at $\Gamma/2 = 0.5$, S. Hietanen and L. G. Sillén, *Acta Chem. Scand.*, 6, 747 (1952). Cl^- and I^- at $\Gamma/2 = 0.5$, L. G. Sillen, *ibid.*, 3, 539 (1949). Br^- at $\Gamma/2 = 0.5$, Y. Marcus, *ibid.*, 11, 599 (1957). SCN^-, K. B. Yatsimirskii and B. D. Tukhlov, *Zhur. obshchei Khim.*, 26, 356 (1956). CN^-, at $\Gamma/2 = 0.1$, G. Anderegg, *Helv. Chim. Acta*, 40, 1022 (1957). Pyridine, at $\Gamma/2 = 0.5$, J. Bjerrum, *Chem. Revs.*, 46, 387 (1950). NH_3, at $\Gamma/2 = 2$, J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941 and 1957, p. 173. Imidazole and histidine (his) at $\Gamma/2 = 0.15$, P. Brooks and N. Davidson, *J. Am. Chem. Soc.*, 82, 2118 (1960). RS^-, at $\Gamma/2 = 1$, W. Stricks and I. Kolthoff, *ibid.*, 75, 5673 (1953). [b] From reference 8. Hughes' values[1b] were slightly higher. [c] Competition with hydroxyl method. [d] pH shift method, [e] Polarography method.

a.—Competition between OH^- and X^- (a base of a strong acid) for CH_3Hg^+— a method due to Waugh, Walton and Laswick,[8] in which one equivalent of CH_3HgOH is titrated with one half equivalent of acid HX in the presence of added salt NaX. At this point

$$\frac{[CH_3HgOH]}{[OH^-]K_3^{OH}} = \frac{[CH_3HgOH]}{[OH^-]10^{9.5}} = [CH_3Hg^+] = \frac{[CH_3HgX]}{[X^-]K_3^X} \quad (7)$$

If $[X^-]K_3^X$ is large enough so that $[CH_3Hg^+]$ is small compared to $[CH_3HgX]$ and small enough so that $[OH^-]$ is small compared to $[CH_3HgOH]$, and if HX is so strong an acid that [HX] is negligible, then $[CH_3HgOH] = [CH_3HgX]$ and $[OH^-] \cdot 10^{9.5} = [X^-]K_3^X$.

Therefore a measurement of pH will allow calculation of K_3^X.

(7) From the wave height the diffusion coefficient calculated on the basis of a one electron reduction was 1.05×10^{-5} cm.²/sec., a value to be expected from comparison with other ions of similar size.

(8) T. D. Waugh, R. E. Walton and J. A. Laswick, *J. Phys. Chem.*, 59, 395 (1955).

b.—Determination of K_3^B by competition between H^+ and CH_3Hg^+ for B^- or B (a base of weak acid). Here the concentration of CH_3Hg^+ is controlled by association with an anion X^- (usually a halide), whose K_3^X already has been determined by method a.

A buffer solution of B^- and HB is made up with added salt to approximately equal the final ionic strength, and the pH is measured. Then a solution containing CH_3HgX and NaX is added and the pH shift noted. The predominant reaction is $CH_3HgX + B^- \rightarrow CH_3HgB + X^-$ and K_3^B is determined from the final concentrations

$$K_3^B = \frac{[CH_3HgB]}{[CH_3Hg^+][B^-]} = \frac{[CH_3HgB]}{[CH_3HgX]} \frac{[X^-][H^+]}{[HB]} K_3^X K_{HB} \quad (8)$$

[HB] remains essentially unchanged during the reaction and the other concentrations (besides $[H^+]$) are obtained by difference. The greatest accuracy is attained if the ratio [B]/[HB] changes from 2 to 1 (from pH = pK_{HB} + 0.3 to pH = pK_{HB}) and if the CH_3HgX added is in excess of the original base B.

In both (a) and (b) the ionic strength was kept close to 0.1 if possible. With some bases, however, the concentration of competing anion had to be higher (up to 1.0) for greater accuracy.

Special Cases of Method b. Cyanide.—Since the complexation with cyanide is so strong, the requirement for greatest accuracy is different from that mentioned above. In a typical cyanide experiment a millimolar solution of KCN was titrated with half an equivalent of HCl (to pH 9.26). Then the addition of one equivalent of CH_3HgCl plus 200 equivalents of KBr decreased the pH to 4.11. Substitution of the final concentrations into equation 8 gives $K_3^{CN} = 10^{14.2}$.

EDTA.—For the determination of the association constant of methylmercury with the triply ionized form of ethylenediaminetetraacetic acid (EDTA) by method b, a solution 4.0 mM in EDTA and 120 mM in chloride was titrated to pH 7.02. An identical solution except for the addition of 4.4 mM CH_3HgCl had pH 6.40. In interpreting these data, the cation binding sites are assumed to be independent (except for electrostatic effects), *i.e.*, the equilibrium constant for replacement of hydrogen by methylmercury is assumed to be independent of the condition of the other sites.

$$\frac{[CH_3HgY^{-3}]}{[HY^{-3}]} = \frac{[CH_3HgHY^{-2}]}{[H_2Y^{-2}]} = \frac{[(CH_3Hg)_2Y^{-2}]}{[CH_3HgHY^{-2}]} \quad (9)$$

where Y represents EDTA.

The main reactions due to the addition of CH_3HgX (at pH 6–7) are

$$CH_3HgX + HY^{-3} \longrightarrow CH_3HgHY^{-2} + X^-$$

$$CH_3HgX + 2HY^{-3} \longrightarrow CH_3HgY^{-3} + H_2Y^{-2} + X^-$$

$$2CH_3HgX + 2HY^{-3} \longrightarrow (CH_3Hg)_2Y^{-2} + H_2Y^{-2} + 2X^-$$

Let $[HY^{-3}]$ and $[H_2Y^{-2}]$ represent concentrations in the absence of CH_3HgCl, *i.e.*, at pH 7.02 $[HY^{-3}] = 3.515$ and $[H_2Y^{-2}] = 0.485$.

In the presence of CH_3HgCl let

$$J = \frac{[HY^{-3}]}{[H_2Y^{-2}]} = \frac{10^{-6.15}}{[H^+]} = \frac{10^{-6.15}}{10^{-6.40}}$$

According to our assumption J is also equal to

$$\frac{[CH_3HgY^{-3}]}{[CH_3HgHY^{-2}]}$$

$$[HY^{-3}] = [HY_0^{-3}] - [CH_3HgHY^{-2}] - 2[CH_3HgY^{-3}] - 2[(CH_3Hg)_2Y^{-2}]$$

$$[H_2Y^{-2}] = [H_2Y_0^{-2}] + [CH_3HgY^{-3}] + [(CH_3Hg)_2Y^{-2}]$$

Combination of the above equations yields

$$[CH_3HgY^{-2}] = \frac{[HY_0^{-3}] - J[H_2Y^{-2}] - (J+2)[(CH_3Hg)_2Y^{-2}]}{(J+1)^2}$$

$$[(CH_3Hg)_2Y^{-2}] = \frac{[CH_3HgHY^{-2}]^2}{[H_2Y_0^{-2}] + J[CH_3HgHY^{-2}] + [(CH_3Hg)_2Y^{-2}]}$$

Solution of these equations by successive approximations gives $[CH_3HgHY^{-2}] = 0.313$ mM and $[(CH_3Hg)_2Y^{-2}] = 0.087$ mM.

$$K_3^{HEDTA} = \frac{[CH_3HgHY^{-2}]}{[HY^{-3}]} \frac{[Cl^-]}{[CH_3HgCl]} K_3^{Cl} = \frac{0.313}{1.93} \frac{120}{3.45} 10^{6.45} = 10^{6.3}$$

Histidine.—Binding to the amine group was measured by pH shift. To a solution of histidine titrated to pH 9.40 in 143 mM Br was added CH_3HgCl and NaBr to give final concentrations of 3 mM histidine, 2.5 mM methylmercury and 150 mM bromide. The pH was measured as 9.06.

$$\text{at } p\text{H } 9.40 \frac{NH_2}{NH_3^+} = \frac{10^{9.40}}{10^{9.10}} = \frac{2.0 \text{ m}M}{1.0 \text{ m}M}$$

$$\text{at } p\text{H } 9.06 \frac{NH_2}{NH_3^+} = \frac{10^{9.06}}{10^{9.10}} = \frac{0.9 \text{ m}M}{1.0 \text{ m}M}$$

The final concentration of CH_3HgNH_2 (his) is equal to the change in NH_2 concentration, 2.0 − 0.9 = 1.1 mM. It is now assumed, as shown below in equation 13, that binding of methylmercury to the imidazole group is negligible at pH 9.06. So the concentration of CH_3HgBr = 2.5 − 1.1 = 1.4 mM. These numbers in equation 8 yield a value of $10^{8.8}$ for the association constant of methylmercury with the amine group of histidine.

c.—Shift of the polarographic half-wave potential.[9] For the reversible reduction of a monovalent cation M^+ whose reduced form is soluble in mercury

$$E_B - E_0 = 59.1 \log \frac{i_0}{i_B} \frac{[M^+]}{[M^+] + [MB] + [MOH] + \ldots} \quad (10)$$

$$= 59.1 \log \frac{i_0}{i_B} \frac{1}{1 + K_3^B[B] + K_3^{OH}[OH^-] + \ldots} \quad (11)$$

$$\cong 59.1 \log \frac{i_0}{i_B} \frac{1}{1 + K_3^B[B]} \quad (12)$$

where i_B and i_0 are the diffusion currents and E_B and E_0 the half-wave potentials of the complexed and free cations, respectively. Conditions are chosen to minimize the complexation due to OH^- and other interfering ions so that equation 11 usually reduces to equation 12.

At 0.1 ionic strength the average value of E_0 for methylmercury calculated (with the aid of the known K_3's) from many experiments with the halides was about −60 mv. Since E_0 varies slightly with ionic strength and other factors, it was

(9) I. M. Kolthoff and J. J. Lingane, "Polarography," Vol. I, 2nd Ed., Interscience Publishers, Inc., New York, N. Y., 1952, p. 214.

determined for each set of polarograms of an "unknown" by a separate polarogram of a halide solution with approximately the same ionic strength. Since the diffusion currents of most of the complexes were nearly equal to those of the standardizing halides, the factor i_0/i_B was ignored in all this work.

The criteria for reversibility are met as well by methylmercury as by the examples[10] cited in "Polarography." The slopes of log $i/(i_d - i)$ *vs.* voltage for almost all methylmercury complexes are between 59 and 63 millivolts, in close agreement with the theoretical value of 59 millivolts. The voltage difference between one fourth and three fourths of the diffusion current is 56 to 60 millivolts, very close to the theoretical value of 56 millivolts.

Since association constants calculated from polarography of the halides, pyridine, imidazole and thiocyanate agreed satisfactorily (within about 0.1 in logs) with those calculated from the other methods, they served only to give some confidence in the polarographic method and are therefore not included in the tables.

Others (acetate, phenolate and cyanide), as Table IV shows, did not give the same value of the association constant at various ligand concentrations. In view of these discrepancies, the only polarographic results included in the main table (Table III) are for the ligands (acetate, phenolate and the imidazole group of histidine) for which the other methods are unsuitable.

TABLE IV

METHYLMERCURY ASSOCIATION CONSTANTS FROM POLAROGRAPHIC HALF WAVE POTENTIALS

Total "ligand" concn. (B + BH)	Ligand	*p*H	Shift in half wave potential $E_B - E_0$ (mv.)	Log of association constant K_3^B	
0.97	Acetate	5.5	−208	K_3^{Ac}	3.6
2.2	Acetate	5.5	−218	K_3^{Ac}	3.4
0.099	Phenolate	9.7	−326	K_3^{Ph0}	6.8
.396	Phenolate	9.7	−332	K_3^{Ph0}	6.3
.792	Phenolate	9.7	−343	K_3^{Ph0}	6.1
.010	Cyanide	4.4	−449	K_3^{CN}	14.5
.010	Cyanide	4.7	−471	K_3^{CN}	14.5
.010	Cyanide	7.05	−607	K_3^{CN}	14.6
.0094	Cyanide	9.2	−756	K_3^{CN}	15.1
.0138	Cyanide	9.8	−762	K_3^{CN}	14.9
.046	Cyanide	9.9	−801	K_3^{CN}	15.0
.23	Cyanide	10.0	−845	K_3^{CN}	15.0
.37	Cyanide	10.0	−857	K_3^{CN}	15.1
.008	$HEDTA^{-3}$	6.5	−280	K_3^{HEDTA}	6.8
.020	$HEDTA^{-3}$	6.5	−300	K_3^{HEDTA}	6.8
.040	$HEDTA^{-3}$	6.5	−317	K_3^{HEDTA}	6.7
.010	Histidine	5.8	−262	$K_3^{Im(his)}$	6.4
.010	Cysteine	2.45	−493	K_3^{RS}	16.6
.0033	Glutathione	3.4	−497	K_3^{RS}	16.5

The causes of the discrepancies are not clear.

The association with acetate is so weak that small amounts of anions diffusing into the polarographic cell from the salt bridge may affect the results.

Possible causes of variation of K_3^{Ph0} may be oxidation of phenol, absorption of phenol on the mercury drop and the appreciable contribution of CH_3HgOH to the shift in half wave.

The slope of the CH_3HgCN wave becomes progressively smaller as the *p*H decreases below 4, indicating irreversibility. Above *p*H 4 the slope is correct, but values of the association constant are considerably higher than $10^{14.2}$ determined by the *p*H shift.

Although other coördination positions of the mercury in CH_3HgCN might be expected to bind additional CN^-, the small variation of K_3^{CN} with a large change in (CN^-) shows that higher complexes are unimportant. The variable and high association constants determined polarographically remain unexplained.

In the polarographic measurement of binding to the imidazole group of histidine, equation 11 must be used because binding to the amine group is also significant. This contribution is calculated from the constant $K_3^{NH_2(his)}$ previously determined by the *p*H shift method.

The use of the polarographically determined $K_3^{Im(his)}$ in

$$\frac{[CH_3HgIm(his)]}{K_3^{Im(his)}[Im(his)]} = [CH_3Hg^+] = \frac{[CH_3HgNH_2(his)]}{K_3^{NH_2(his)}[NH_2(his)]} \quad (13)$$

shows that under the final conditions of the histidine *p*H shift experiment, binding to imidazole was only a hundredth of that to amine.

In the polarography of EDTA (Y), the concentrations of Y^{-4} and $(CH_3Hg)_2Y^{-2}$ were negligible. The same assumption made in equation 9 that the cation binding sites are independent leads to the relation

$$K_3^{HEDTA} = \frac{[CH_3HgHY^{-2}]}{[CH_3Hg^+][HY^{-3}]} = \frac{[\text{total } CH_3Hg]}{[CH_3Hg^+][\text{total Y}]}$$

Spectrophotometry.—A confirmation of the correctness of the relative values of K_3^{CN} and K_3^{RS} was obtained by measuring spectrophotometrically the competition between cyanide and sulfhydryl for paracarboxy-phenylmercuric chloride (para-chloromercuribenzoate, PCMB). As Boyer showed,[11] there is a large increase in optical density of PCMB when mercaptide is formed. If $\Delta\epsilon = 0$ with no mercaptide and 1 with stoichiometric formation of mercaptide, then an equation for PCMB similar to this one for methylmercury

$$\text{Ratio} = \frac{K_3^{RS}}{K_3^{CN}} = \frac{K_{RSH}}{K_{HCN}} \frac{[CH_3HgSR][HCN]}{[CH_3HgCN][RSH]} \quad (14)$$

yields (with 10^{-4} *M* PCMB and 10^{-4} *M* cysteine in various concentrations of HCN)

$$\text{Ratio} = \frac{K_{RSH}}{K_{HCN}} \frac{[10^{-4}\,\Delta\epsilon][HCN]}{[10^{-4}(1 - \Delta\epsilon)][10^{-4}(1 - \Delta\epsilon)]} \quad (15)$$

There is also some absorption due to HCN, and this must be subtracted to obtain the filled circles in Fig. 1 agreeing with equation 15. The best fit is with a ratio = $10^{1.7}$, only $10^{0.1}$ larger than the ratio calculated for methylmercury from the constants in Table III. Although Fig. 1 is for *p*H 3.8, the same value of the ratio was obtained in a phosphate buffer at *p*H 6.7.

(10) I. M. Kolthoff and J. J. Lingane, *J. Phys. Chem.*, pp. 193–194, 228.

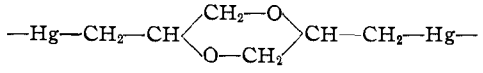
(11) P. D. Boyer, *J. Am. Chem. Soc.*, **76**, 4331 (1954).

Discussion

Equation 2 enables us to calculate the association constant K_3^{RS} of methylmercuric ion with the ionized form of sulfhydryl or to calculate $K_2 = K_3^{RS}/K_{RSH}$, a more convenient quantity for comparison of model compounds with proteins in which K_{RSH} is not known. From Tables I, II and III, $K_2 = K_1^{Br}D_{Br}K_3^{Br} = 10^{-1.26} \times 45 \times 10^{6.7} = 10^{7.1}$ for cysteine and $10^{-1.45} \times 45 \times 10^{6.7} = 10^{6.9}$ for glutathione. Hughes[1a] had measured K_1^I (where I refers to iodide) as $10^{-4.5}$ for human mercaptalbumin and within $10^{0.1}$ of this for cysteine. This value and the appropriate values for iodide from Tables II and III yield $K_2 = 10^{-4.5} \times 500 \times 10^{3.7} = 10^{6.9}$, in quite satisfactory agreement with the values above, and with the value 10^7 published by Hughes.[1b]

At about 0.1 ionic strength the association constants K_{RSH} of hydrogen ion with the sulfhydryl group[12] in cysteine and glutathione are $10^{8.6}$ and $10^{9.0}$ (1./mole), respectively, from which K_3^{RS} is calculated as $10^{15.7}$ and $10^{15.9}$ (1./mole), *i.e.*, within experimental error.

Albumin Dimer with Mercurial.—ASHgR'HgSA, the dimer of human mercaptalbumin with the bifunctional organic mercurial



may be considered as the mercaptide of ASHgR'-Hg^+ and AS^-. Its dimerization constant K_A is therefore also a mercaptide association constant similar to equation 1. A constant independent of the unknown sulfhydryl pK, K_2' (similar to K_2 of equations 2 and 6) is defined as

$$K_2' = \frac{K_A}{K_{RSH}} = \frac{[ASHgR'HgSA][H^+]}{[ASHgR'Hg^+][ASH]} \qquad (16)$$

K_2' had been estimated[13] as $10^{8.2}$ from dimerization in the presence of cyanide.

$$K_2' = \frac{K_{A1}K_{A_2}^{CN}}{K_{HCN}} \qquad (17)$$

where

$$K_{A_1} = \frac{[ASHgR'HgSA][HCN]}{[ASHgR'HgCN][ASH]} \cong 1 \qquad (18)$$

$$K_{A_2}^{CN} = \frac{[ASHgR'HgCN]}{[ASHgR'Hg^+][CN^-]} \cong 10^{17.5} \qquad (19)$$

$$K_{HCN} = \frac{[HCN]}{[H^+][CN^-]} = 10^{9.3} \qquad (20)[14]$$

but this is incompatible with $K_2 = 10^7$ and a light scattering experiment[15] which showed that equilibrium in the reaction

$$CH_3HgCl + ASHgR'HgSA \longrightarrow CH_3HgSA + ASHgR'HgCl \qquad (21)$$

favors the right hand side. It seems reasonable to suppose that the affinity of the two mercurials

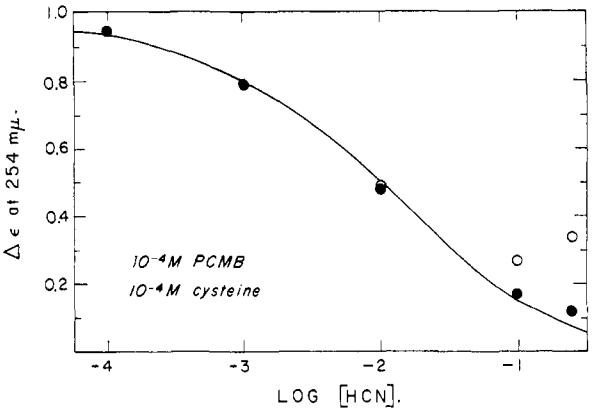


Fig. 1.—Effect of HCN on the increase in absorption at 254 mμ of 10^{-4} PCMB + 10^{-4} cysteine (over that of PCMB alone). O, uncorrected; ●, corrected (for HCN) by subtracting optical density of same solution without cysteine. Curve calculated from equation 15. Measurements in 0.1 ionic strength acetate pH 3.8, O.D. is 0.60 at $\Delta\epsilon = 0$, and 1.15 at $\Delta\epsilon = 1$.

(ASHgR'Hg^+ and CH_3Hg^+) for chloride is practically the same and therefore that $K_2/K_2' > 1$. It therefore seems likely that the former estimate of K_2' was too high. Although the other two constants used in calculating K_2' were based on direct experiment, $K_{A_2}^{CN}$ was estimated by analogy on the tentative assumption that it could be taken equal to the constant for $\sqrt{[Hg(CN)_2]/[Hg^{++}][CN^-]^2}$. A closer analogy would obviously be the combination of the organic mercurial itself with cyanide, but the very low solubility of the halides and cyanide of the organic mercurial (about 10^{-4} M) thwarted attempts to determine the association constants (by the method of pH shift). The association constant of methylmercury with cyanide K_3^{CN} should, however, be not far different from $K_{A_2}^{CN}$ since here too there is an aliphatic group attached to the mercury. If the value of K_3^{CN}, $10^{14.2}$, is used instead of $10^{17.5}$, as an approximation for $K_{A_2}^{CN}$ the value of K_2' is compatible with the

$$K_2' = \frac{K_{A_1}K_{A_2}^{CN}}{K_{HCN}} \cong \frac{1 \times 10^{14.2}}{10^{9.3}} \cong 10^{4.9} \qquad (22)$$

equilibrium in equation 21. If the same estimate (10^{10}) of the association constant of the sulfhydryl group of mercaptalbumin is made as in reference 13, then the equilibrium constant for dimerization with the organic mercurial

$$K_A = \frac{[ASHgR'HgSA]}{[ASHgR'Hg^+][AS^-]} \qquad (23)$$

is also reduced by the factor $10^{14.2}/10^{17.5}$ to the value $10^{14.9}$

Albumin Dimer with Mercury.—The equilibrium constants $K_A(Hg) = [ASHgSA]/[ASHg^+][AS^-]$ for the dimerization of human albumin and of bovine albumin with mercury probably should be revised to smaller values too, since the estimates[13,16] of these constants employed an analogy similar to that used in estimating $K_{A_2}^{CN}$. The affinity of $ASHg^+$ for Cl^- was assumed to be equal to that of $HgCl^+$ for Cl^- ($K = 10^{6.5}$). If we assume instead

(12) L. R. Ryklan and C. L. A. Schmidt, *Arch. Biochem.*, **5**, 89 (1944). R. Benesch and R. E. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955). M. A. Grafius and J. B. Neilands, *ibid.*, **77**, 3389 (1955). R. B. Martin and J. T. Edsall, *Bull. Soc. Chim. Biol.*, **40**, 1763 (1958).

(13) J. T. Edsall, R. H. Maybury, R. B. Simpson and R. Straessle, *J. Am. Chem. Soc.*, **76**, 3131 (1954). The dimerization was measured at pH 4.75 in acetate buffer at 25°. The pH dependence of dimerization, measured with the mercury dimer, has not been quantitatively explained.

(14) N. V. Sidgwick, ref. 2, Vol. I, p. 670.

(15) R. Straessle, personal communication.

(16) C. M. Kay and J. T. Edsall, *Arch. Biochem. Biophys.*, **65**, 375 (1956).

that $ASHg^+$ has the same affinity for Cl^- as CH_3Hg^+ does ($K_3^{Cl} = 10^{5.45}$), the equilibrium constant for the mercury dimer of human mercaptalbumin would be reduced from $10^{13.6}$ to $10^{12.5}$. A similar recalculation involving bromide reduces the former estimate of $K_A(Hg)$ from $10^{13.4}$ to $10^{11.8}$. That the discrepancy between $10^{12.5}$ and $10^{11.8}$ is larger than we would expect from experimental error emphasizes the roughness of our assumption that $ASHg^+$ is like CH_3Hg^+. Nevertheless a value of about 10^{12} for $K_A(Hg)$, where K_{ASH} is again taken as 10^{10}, seems more reasonable than the previous estimate. Thus the repulsion between the albumin molecules reduces the constant for this mercaptide about 10^5 below that[17] for methylmercury reacting with human mercaptalbumin.

The value of $K_A(Hg)$ for bovine[16] mercaptalbumin is about an order of magnitude larger than for human, whereas the opposite order is found[1b] for the strength of the methylmercury mercaptides. These data could be reconciled by supposing that in the human albumin some local (electronic) effect makes the Hg–S bond intrinsically stronger but that the over-all conformation makes dimer formation more difficult.

Other Comments.—Equilibrium in equation 21 should be the same as in the reaction

$$ASHgR'HgSA + XHgR'HgX = ASHgR'HgX + XHgR'HgSA \quad (24)$$

if, as seems likely, the state of equilibrium in each case (with one mole of mercurial added to one mole of dimer) differs from a statistical distribution of AS^- and X^- groups on the available mercury sites only because of the repulsion between albumin molecules of dimer. Although equilibrium in equation 21 was not accurately measured directly, this equilibrium constant is calculated (with the assumption mentioned after that equation) as $K_2/K_2' = 10^{7.0}/10^{4.9} = 10^{2.1}$. This value does not seem too far from the equilibrium constant $K_B = 10^{3.0}$ of equation 24 in view of the large effect of experimental error.[18]

An unexplained observation on the mercurial dimer invites speculation. In an acetate buffer at *p*H 4.75 with one mole of mercurial and two moles of mercaptalbumin at a high enough concentration that almost complete dimerization would be expected, light scattering indicates only about 0.86 weight fraction dimer for human mercaptalbumin.[13] Let us make the hypothesis that one of the nitrogen base groups, let us say an imidazole, of the albumin is located at a short distance from the sulfhydryl group so that formation of $\overline{ASHgR'HgN}$ is facilitated. (In the following discussion only this imidazole is considered.) To form the dimer, the sulfhydryl group of the second albumin has to compete with this imidazole for the free mercury valence of $ASHgR'Hg^+$. The equation for the competition is

$$\frac{(ASHgR'HgSA)}{(AS^-)K_A} = (ASHgR'Hg^+) = \frac{(\overline{ASHgR'HgN})}{\left(\begin{matrix}\text{Concentration of imidazole free}\\ \text{to combine with mercury valence}\end{matrix}\right)K_4}$$

where K_A is the dimerization constant and K_4 is defined as the association constant of $ASHgR'Hg^+$ with the assumed nearby imidazole.

In the example above with 0.86 weight fraction dimer the relative concentrations are (ASHgR'HgSA) = concentration of imidazole free = $\frac{1}{2} \times 0.86 = 0.43$ and $(AS^-) = (\overline{ASHgR'HgN}) = \frac{1}{2}(1.00 - 0.86) = 0.07$. Substitution in the above equation yields $K_A = 10^{1.6} K_4$. The model compounds in Table III show that in the absence of steric factors the sulfhydryl would ordinarily have an association constant with an organic mercurial about 10^6 times that of the imidazole. The discussion following equation 20 showed that the affinity of the second albumin for the dioxane mercurial is smaller by a factor of $10^{-2.1}$ to $10^{-3.0}$ than that of the first albumin or of a typical unhindered sulfur-organic mercurial bond. If we take $10^{2.5}$ as an average for this factor, K_4 must be larger than the ordinary association constant of mercurial with imidazole by a factor roughly $\times 10^{6.0}\, 10^{-2.5} \times 10^{-1.6} = 10^{1.9}$. This is a plausible value for the "steric assistance" we have assumed. This explanation of the data at *p*H 4.75 is inconsistent with the observation[13] of complete dimerization at *p*H 5.5 and *p*H 6.0 unless we make an additional hypothesis, *e.g.*, that between *p*H 4.75 and 5.5 a carboxyl group becomes ionized and forms a hydrogen bond with the imidazole.

Some comments on other values in Table III may be made. Sidgwick[19] has pointed out the low affinity of mercury for oxygen. If the competition between the ions of mercury and hydrogen for bases is considered, Table III shows that hydrogen has a much greater affinity than mercury for bases with an oxygen (except for EDTA), that mercury has a much greater affinity for bases with a sulfur and that they compete approximately equally for nitrogen bases.

If the constants in Table III can be considered as applicable to the functional groups of protein then at low *p*H methylmercury would first saturate the sulfhydryl groups, and larger amounts would be distributed between (equal numbers of) imidazole and amine groups in a ratio of about 4 to 1. As the *p*H increased above 6, the binding to amine groups would continue to increase, while binding to imidazole groups would level off. (The binding to guanidine groups probably would be small.)

The greater affinity of methylmercury for EDTA than would be predicted from its behavior with acetate suggests chelation involving the third and fourth coördination positions of mercury. The chelation must be weak, however, since constants from the references in Table III show that binding

(17) From the value $K_2 = 10^{7.0}$ in the first paragraph of the discussion and the same assumed value of pK 10 for the albumin sulfhydryl.

(18) On page 3136 of ref. 13 in the determination of K_B, the concentration of the dimer should have been half the concentration of albumin in dimer, *i.e.*, 0.04 instead of 0.08, so that K_B is correctly calculated as $10^{3.0}$ instead of $10^{2.4}$. This change is not very significant, however, since an error of only 3% in the light scattering measurement upon which the calculation is based would change K_B by as much as this arithmetic error.

(19) N. V. Sidgwick, ref. 2, Vol. I, p. 300.

to the third and fourth coördination positions is from 10^5 (for chloride) to 10^{14} (for cyanide) weaker than to the first two. It should be noted also that the ratio of the association constant of methylmercury to that of hydrogen for EDTA is much less than this ratio for other metals, except the alkali metals and alkaline earths. Therefore EDTA is useful as a complexing agent to remove interfering metals in studies of mercury-sulfhydryl association.

[CONTRIBUTION FROM THE EVANS LABORATORY OF CHEMISTRY, THE OHIO STATE UNIVERSITY, COLUMBUS, OHIO]

A Cryoscopic Study of the Diammoniate of Diborane in Liquid Ammonia[1]

BY B. Z. EGAN AND S. G. SHORE

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Molecular weight studies on the diammoniate of diborane, $[H_2B(NH_3)_2][BH_4]$, in liquid ammonia, using a freezing point depression technique to a precision of $\pm 0.005°$ indicate that the system is well behaved. Observed concentration dependence is characteristic of a salt. Anomalous concentration dependence observed in previous investigations, using vapor pressure depression methods was not observed. No evidence was found for the existence of a higher molecular weight substance such as $[HB(NH_3)_3][BH_4]_2$, which has been previously postulated to form in solutions allowed to age at $-45°$ or higher for extended periods of time. Comparison of the results of this investigation with that of Rathjens and Pitzer indicates that the initial reaction of diborane with ammonia may lead to non-dissociated two boron atom species.

Introduction

Although the structural formula of the diammoniate of diborane seems to be well established as $[H_2B(NH_3)_2][BH_4]$ through both physical and chemical studies,[2] there exist some unsolved problems with respect to its nature in liquid ammonia. From reactions with sodium in liquid ammonia and molecular weight studies in liquid ammonia, determined through vapor pressure depression techniques, it would seem as though another species of higher apparent molecular weight is formed upon prolonged standing of the solution at temperatures of $-45°$ or higher. One of the structures proposed for the "high temperature form" is $[BH(NH_3)_3][BH_4]_2$. This structure previously has been referred to as "diammoniate II."[3,4] However, later studies by Parry and Kodama[5] and Parry and Shore[5] raise some serious questions with respect to the existence of the "diammoniate II." Furthermore, careful examination of the existing molecular weight data presents some problems in rationalization. First, the molecular weight seems to rise with increasing dilution, even at concentrations where solvation effects would not seriously affect the observed apparent molecular weight. Secondly, the data seem to distinguish between high and low molecular weight species, yet the data were obtained over extended periods of time, at temperatures presumed to be conducive to the formation of the higher molecular weight material. There was no evidence that a specific sample of specific concentration, which had a low apparent molecular weight initially, was converted under the conditions of the study to the higher molecular weight form.

Because of the uncertainty in molecular weight studies, it seemed desirable to examine again the apparent molecular weight of the diammoniate of diborane in liquid ammonia. A freezing point technique was used in which freezing point depressions could be determined to a precision of about $\pm$ 0.005°. Measurements were made more rapidly and at lower temperatures than in the vapor pressure depression method. It was possible to determine the apparent molecular weight of a sample of "authentic" diammoniate of a given concentration which had not been above $-78°$ and then observe whether storing the solution at $-45°$ for an extended period of time had an effect on the apparent molecular weight. Also, through the use of X-ray powder diffraction techniques, it was possible to determine, at room temperature, whether solids isolated from solutions stored at $-45°$ or higher differed in crystalline nature from solids isolated from solutions stored at $-78°$.

Experimental

A. Materials.—1. Diborane was prepared and purified according to well known methods.[6] 2. Ammonia was dried over sodium and then distilled into a weighing bulb containing anhydrous lithium nitrate which had previously been heated to 160° and pumped on for a period of one week. The saturated solution of lithium nitrate in ammonia had a vapor pressure of less than one atmosphere at room temperature. Thus, a desired amount of ammonia, of the order of several grams, could be distilled from the bulb which had been weighed at room temperature. 3. Diammoniate of diborane was prepared according to a set of carefully prescribed conditions.[3]

B. Apparatus.—1. Standard high vacuum techniques were used for handling materials. 2. A freezing point cell[7] was used in conjunction with a Mueller Type G-2 Temperature Bridge and a calibrated four lead, capsule platinum resistance thermometer. The resistance could be read directly to 0.0001 ohm. On the particular thermometer used, this corresponded to about 0.001°.

C. Experimental Procedure.—About 10 g. of ammonia, weight known to ± 0.5 mg., was condensed into the freezing point cell. The ammonia was warmed to a temperature

(1) Presented before the Inorganic Division at the 139th Meeting of the American Chemical Society, St. Louis, Missouri, March, 1961.

(2) R. W. Parry, P. R. Girardot, D. R. Schultz and S. G. Shore, *J. Am. Chem. Soc.*, **80**, 1 (1958); R. C. Taylor, D. R. Schultz and A. R. Emery, *ibid.*, **80**, 27 (1958); T. P. Onak and I. Shapiro, *J. Chem. Phys.*, **32**, 952 (1960).

(3) R. W. Parry and S. G. Shore, *J. Am. Chem. Soc.*, **80**, 15 (1958).

(4) R. W. Parry, G. Kodama and D. R. Schultz, *ibid.*, **80**, 24 (1958).

(5) R. W. Parry, G. Kodama and S. G. Shore, WADC Technical Report 59-207.

(6) I. Shapiro, H. G. Weiss, M. Schmich, S. Skolnik and G. B. L. Smith, *J. Am. Chem. Soc.*, **74**, 901 (1952).

(7) Details of the construction of this cell can be obtained from S. G. Shore, Department of Chemistry, The Ohio State University, Columbus 10, Ohio.