

Fig. 3.—Iodobenzene in: 1, cyclohexane; 2, 95% ethanol; 3, water containing a minimum amount of methanol.⁴ Concentration at the primary band $3-5 \times 10^{-6}$ mole per liter, at the secondary band $3-6 \times 10^{-4}$ mole per liter. The fine structure bands required measurements at 0.5 μ intervals.

for the smaller water molecules.¹⁷ The blue shifts

(17) It is necessary to assume a hydrate involving the unshared electron pair in aromatic amines in order to account for the basic properties of these substances. The formation of such hydrates is also subject to steric effects (H. C. Brown, *Science*, **103**, 385 (1946); *This Journal*, **67**, 374, 378 (1945)).

in iodobenzene are actually more pronounced than the values for λ_{\max} would indicate because the primary band shows asymmetric broadening in alcohol and appears as a doublet in cyclohexane (Fig. 3).

A tentative explanation of the experimental results is offered on the basis of the present data. A blue shift of the primary band caused by increasing the polarity of the solvent implies a decrease in the polar quinoid resonance forms. The benzene substituents in these compounds are electron donors, characterized by unshared electron pairs, which may interact with the respective nuclei and partake in hydrogen bonds with the solvent (solvation) or with other molecules (association). Blue shifts would be accounted for by assuming that the solvation with polar solvents contributes to the stability of the ground states more than it does to the excited states.¹⁸ Barring other effects, the blue shifts should increase progressively with the solvent polarity. This appears to be the case for phenyl ethers, dimethylaniline and iodobenzene, while abnormal solvent sequences are observed for phenol, aniline and acetanilide, *i.e.*, in substances in which association is known to occur.

The effect of a solvent is apparently dependent on the degree and type of solvent bonding (or dipole association), or, particularly in non-polar solvents, on the degree of association. It is well known that such molecular interaction is sensitive to steric effects. A more detailed study would be indicated in order to establish what other factors need to be considered in order to evaluate solvent effects.

Acknowledgment.—The author is indebted to Dr. J. R. Platt for valuable suggestions.

(18) The suggestion of a ground state stabilization is not original since it was proposed by L. G. S. Brooker and R. H. Sprague (*ibid.*, **63**, 3215 (1941)) to explain the lack of color in Michler ketone "even in strongly polar solvents."

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The Consecutive Constants for the Association of Cadmium with Imidazole^{1,2}

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The successive association constants of the cadmium-imidazole complex have been determined potentiometrically by the method of Bjerrum. At 25° $\log k_1 = 2.80$, $\log k_2 = 2.10$, $\log k_3 = 1.55$ and $\log k_4 = 1.13$. From measurements at other temperatures ΔH_1 is estimated to be -5 ± 2 kcal./mole. The value of $\log k_1$ is identical with the logarithm of the intrinsic association constant of cadmium with the imidazole groups of serum albumin.

Recent work from this Laboratory and elsewhere^{3,4} has indicated that in the combination of metals with serum albumin the principal binding sites are the imidazole groups of the protein molecule. If this is so the binding constant for the reaction of a metal with imidazole itself should

be of the same order of magnitude as the intrinsic constant found for the association of the same metal with serum albumin. The present paper verifies this conclusion for cadmium. A similar verification for zinc has been made by Gurd and Goodman.⁴

Experimental

The reaction was studied potentiometrically by the method of Bjerrum.⁵ A Beckman model G pH meter with external electrodes was used, and the procedure employed

(1) This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

(2) Abstracted from a thesis by Myron L. Wagner in partial fulfillment of the requirements for the M.S. degree, State University of Iowa, June, 1952.

(3) C. Tanford, *This Journal*, **74**, 211 (1952).

(4) F. R. N. Gurd and D. S. Goodman, *ibid.*, **74**, 670 (1952).

(5) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

was virtually identical with that used by Bjerrum for study of the formation of cadmium amines, except that a lower ionic strength (0.15) was used. The determinations were made at three temperatures, 15, 25 and 35°, with nitrate as the only anion present.

Imidazole was obtained from Edcan Laboratories and purified by distillation at low pressure, followed by recrystallization from dry benzene. C.P. cadmium nitrate was used to make a stock solution which was analyzed by conversion to CdSO_4 .

Results and Discussion

The hydrogen ion dissociation constant of the imidazolium ion, required for application of the Bjerrum method, was obtained from complete titration curves at ionic strength 0.15. The pK values obtained are 7.31, 7.12 and 6.93, respectively, at 15, 25 and 35°, with a probable error of ± 0.02 . These lead to values of 7.5 kcal./mole and -6.7 e.u., respectively, for the heat and entropy of ionization.

The formation curves for the cadmium-imidazole complexes are shown in Fig. 1. The data at 25° are not shown to avoid crowding the figure. All the points at that temperature fall between those at 15 and 35°. The curves have been extrapolated to a maximum of 4 imidazoles bound, but it is quite possible that 6 may be bound, as in the case of cadmium amines.⁵ In any event only the first three association constants can be computed from Fig. 1 with any accuracy. Constants so computed are shown in Table I. They were calculated in exactly the same manner as was used by Bjerrum for cadmium amines. The probable accuracy is ± 0.05 for $\log k_1$, ± 0.08 for $\log k_2$ and ± 0.15 for $\log k_3$. Values of $\log k_4$ were estimated from the extrapolated portions of the curves of Fig. 1. Their accuracy is estimated to be ± 0.2 .

The constants of Table I refer to ionic strength 0.15. However, the difference between them and the corresponding true activity constants is likely to be smaller than the experimental error.

TABLE I
CONSECUTIVE ASSOCIATION CONSTANTS^a

	15°	25°	35°
$\log k_1$	2.88	2.80	2.62
$\log k_2$	2.18	2.10	2.03
$\log k_3$	1.53	1.55	1.55
$\log k_4$	1.17	1.13	0.96

^a These constants refer to the reactions $\text{Cd}^{++} + \text{Im} \rightleftharpoons \text{CdIm}^{++}$; $\text{CdIm}^{++} + \text{Im} \rightleftharpoons \text{CdIm}_2^{++}$; etc.

From the values of $\log k_1$, ΔH_1 , i.e., ΔH for the binding of the first imidazole molecule, is found to be -5 ± 2 kcal./mole. The data are not precise enough to permit an accurate determination of the corresponding entropy change: however, the value is certain to be small, as would be expected for a

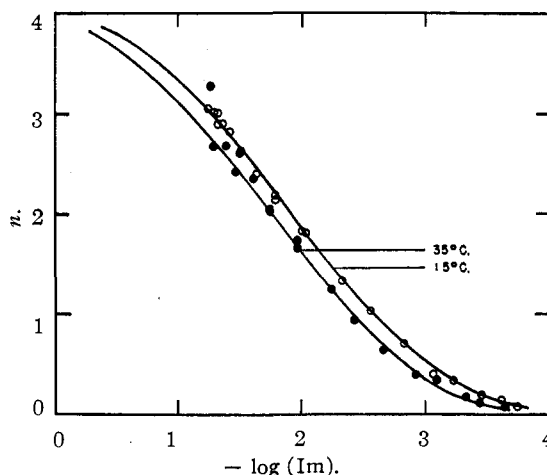


Fig. 1.—Formation curves for cadmium-imidazole complexes. The average number of ligands per Cd^{++} ion is plotted against the logarithm of the concentration of free uncharged imidazole.

reaction which consists merely of the displacement of a coordinated water molecule by imidazole.

The value of $\log k_1$ obtained in this work is, within the experimental error, identical with the intrinsic association constant obtained for the combination of cadmium ion with the imidazole groups of bovine serum albumin ($\log k = 2.8 \pm 0.2$). It appears, therefore, that the uncharged imidazole groups of serum albumin are not concerned in any internal bonding and that their combination with cadmium ion does not involve joint action with other groups on the protein molecule.

Another observation of interest lies in the similarity between imidazole and ammonia in their affinity for cadmium and zinc (Table II). Not only are the constants very close in magnitude, but the same difference is observed between cadmium and zinc in both cases: the successive constants for the former drop off rapidly, whereas for zinc they do so much more slowly. No reason for this difference is known.

TABLE II
COMPARISON OF IMIDAZOLE WITH AMMONIA

	Cd-NH_3^a	Cd-Im^b	Zn-NH_3^a	Zn-Im^c
$\log k_1$	2.51	2.80	2.18	2.76
$\log k_2$	1.96	2.10	2.25	2.38
$\log k_3$	1.30	1.55	2.31	2.40
$\log k_4$	0.79	1.13	1.96	2.20

^a J. Bjerrum, ref. 5, p. 163, ionic strength zero, 30°. ^b This paper, ionic strength 0.15, 25°. ^c F. R. N. Gurd, J. T. Edsall, G. Felsenfeld and D. S. Goodman, *Federation Proc.*, 11, 224 (1952); ionic strength 0.16, 4.5°.

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