(21) R. Grigg, G. Shelton, A. Sweeney, and A. W. Johnson, *J. Chem. Soc., Perkin Trans. 1*, 1789 (1972).
(22) H. Ogoshi, E. Watanabe, N. Koketsu, and Z. Yoshida, *J. Chem. Soc., Chem. Commun.*, 943 (1974).
(23) J. H. Weber and G. N. Schrauzer, *J. Am. Chem. Soc.*, **92**, 726 (1970).
(24) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(25) J. E. Maskasky and M. E. Kenney, *J. Am. Chem. Soc.*, **93**, 2060 (1971); J. E. Maskasky, J. R. Moorey, and M. E. Kenney, *ibid.*, **94**, 2132 (1972), J. E. Maskasky and M. E. Kenney, *ibid.*, **95**, 1443 (1973).
(26) N. Datta-Gupta, *J. Inorg. Nucl. Chem.*, **33**, 4219 (1972).
(27) H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Discuss. Faraday Soc.*, **47**, 165 (1965).

Thermodynamic Data for the Binding of Molecular Oxygen to Cobalt(II) Protoporphyrin IX Dimethyl Ester

Tony J. Beugelsdijk and Russell S. Drago*

Contribution from the W. A. Noyes Laboratory, University of Illinois, Urbana, Illinois 61801. *Received March 5, 1975*

Abstract: Obtaining accurate thermodynamic data for the reversible oxygenation of transition metal ion complexes is critical to an eventual understanding of the factors that influence the strength of binding. The various amine complexes of cobalt(II) protoporphyrin IX dimethyl ester provide a system in which some interesting variations can be examined quantitatively. Thermodynamic results reported by Ibers et al. on this system have been the subject of recent controversy. The conclusions drawn from our method of analysis of the reported data were rejected and our methods criticized. In this paper, we defend our method of analysis and report new experimental data for the O_2 adduct of the pyridine complex of cobalt(II) protoporphyrin IX dimethyl ester under elevated oxygen pressures. Our results differ markedly from those previously reported by Ibers et al. and verify the conclusions arrived at in our earlier analysis.

The binding of molecular oxygen to transition metal complexes in nonaqueous media has been the subject of much current research.[1] By measuring the equilibrium constant at various different temperatures, the corresponding thermodynamic quantities for binding of O_2 can be calculated. In many of the systems reported in the literature (see ref 2a, for example), a great deal of the chemical information is manifested in 1-2 kcal mol^{-1} enthalpy differences. In order to compare one set of data to another, when some chemical variation is made, it is thus necessary that each set be properly evaluated using rigorous procedures for analyzing and reporting experimental quantities. Without such an evaluation, any correlations of these data to other observables becomes at most stochastic if not meaningless. In a previous paper,[2b] we outlined a basic approach to this problem by stating definite criteria for acceptability of calculated parameters from experimental observables. This approach combines not only the objective results of a statistical analysis but also incorporates a time-tested and experience-proven empirical (i.e., common sense) evaluation of one's data to ensure that the values obtained are not the result of the computer converging to a false minimum. Both these criteria have been explicitly stipulated[2b] and, moreover, the combination of both the objective and empirical elements are more desirable than either one or the other by itself.

Statement of the Problem

The fact that the extinction coefficients for the oxygenated and unoxygenated complexes differ permits the equilibrium constant for oxygen uptake to be determined spectrophotometrically. The equilibrium in question is given by

$$\text{py}\cdot\text{CoPIXDME} + O_2(g) = \text{py}\cdot\text{CoPIXDME}\cdot O_2 \quad (1)$$

where py is pyridine and CoPIXDME is cobalt(II) protoporphyrin IX dimethyl ester. The equilibrium constant expression for this system can be written as

$$K = C_C/(C_A - C_C)P_{O_2} \quad (2)$$

where C_A is the initial molar concentration of acid, C_C the *equilibrium* molar concentration of complex, and P_{O_2}, the equilibrium *partial* pressure of oxygen above the solution. Since both the quantities C_C and $(C_A - C_C)$ can be expressed as a percentage of the initial acid concentration, the fraction of acid complexed is to a good approximation, independent of the total acid concentration. Hence, changes in the concentration of acid due to solvent contraction and variations in the molar absorption coefficients over the temperature range studied are of no consequence. This feature is inherent in any 1:1 equilibrium where the concentration of the varied reagent is measured at equilibrium and not determined knowing initial conditions.

An equilibrium constant expression for a 1:1 adduct in terms of measured absorbances has been previously reported[3] as

$$K^{-1} = \frac{A - A^0}{\epsilon_C - \epsilon_A} - C_A - C_B + \frac{C_B C_A}{A - A^0}(\epsilon_C - \epsilon_A) \quad (3)$$

where C_B = initial base concentration in molarity, ϵ_A = molar absorptivity of the acid, ϵ_C = molar absorptivity of the complex, A^0 = absorbance at a given wavelength due to the initial concentration, i.e., $C_B = 0$, and A = absorbance of system at a given wavelength. The only assumption in the derivation of eq 3 is that there are at most two absorbing species (the acid and the complex) which obey Beer's law in the concentration range employed. This equation is readily converted to eq 4 for measurements on the system described in eq 1

$$K^{-1} = P_{O_2}\left[\frac{C_A b(\epsilon_C - \epsilon_A)}{A - A^0} - 1\right] \quad (4)$$

where K is the equilibrium constant associated with eq 1, P_{O_2} is the partial pressure of oxygen above solution, C_A is the initial concentration of acid (total porphyrin concentration) in moles per liter, b is the path length of the absorption cell, A is the total absorbance which is related to the concentration of the oxygen adduct and to the free acid concentration, A^0 is the absorbance of the solution before admitting any oxygen, and ($\epsilon_C - \epsilon_A$) is the difference between molar absorption coefficients of the complex and free acid, respectively. Since eq 3 contains two unknowns, namely K and ($\epsilon_C - \epsilon_A$), measurement of the solution absorbance at two different pressures will yield a unique solution for both unknowns. Upon solving any pair of such equations (denoted by i and j), for K we obtain

$$K^{ij} = \frac{[(A^i - A^0)/P_{O_2}{}^i] - [(A^j - A^0)/P_{O_2}{}^j]}{A^i - A^j} \qquad (5)$$

In any real experiment, however, errors in the measurement of observables preclude a unique solution to eq 5 and discrepancies result among random pair-wise solutions. The experiment must be so executed as to overdetermine the unknowns and a best average solution reported which is consistent with all these equations in a least-squares sense. It is this solution along with the interpretation of the marginal and conditional deviations which result that constitutes our recently maligned empirical evaluation.[4] In this article, we shall (1) present new data which conclusively justify our earlier conclusions, (2) present a logical rationale for using the method we employ, and (3) indicate the shortcomings of the limiting spectral approach as applied to the O_2 adducts.[4-6]

Experimental Section

Chemicals. Protoporphyrin IX dimethyl ester was prepared from beef blood[7] according to the procedure outlined by Grinstein.[8] Recrystallization was from methanol with a minimal amount of chloroform. Anal. Calcd for $C_{36}H_{38}N_4O_4$: C, 73.19; H, 6.49; N, 9.49. Found, C, 72.23; H, 6.50; N, 9.25. Cobalt(II) protoporphyrin IX dimethyl ester was prepared by the method described by Falk[9] and recrystallized from benzene. Anal. Calcd for $CoC_{36}H_{36}N_4O_4$: C, 66.77; H, 5.60; N, 8.65; Co, 9.10. Found: C, 66.26; H, 5.60; N, 8.34; Co, 9.05. Pyridine was refluxed over and distilled from barium oxide powder. Toluene was refluxed over and distilled from calcium hydride. Prior to making up the solution for study, the toluene was degassed by the freeze-pump-thaw method for a minimum of three cycles and then allowed to warm under vacuum back to room temperature.

Ligand Binding Measurements. All electronic spectra were recorded on a Cary Model 14 RI recording spectrophotometer. The scan rate was at 1 nm sec^{-1}.

High Pressure Cell. The high pressure cell used in this study is similar to the one described by Lakowicz.[10] It was burst tested and found to withstand internal pressures in excess of 1500 psi (102 atm) of oxygen—well above that required in an actual experiment. Oxygen pressure was measured with a Roylyn precision gauge (0-1500 psi) accurate to ±0.25%. The gas handling system is illustrated schematically in Figure 1. The check valve (Hoke, 6233G4S) and pressure relief valve (Hoke 656L4Y) were supplied by Enpro Incorporated of Villa Park, Ill. Both were specified to be cleaned for oxygen service. The purge valve (Nupro SS-4P-4) was supplied by Lakeview Valve and Fitting Co., in Worth, Ill., and was also specified to be cleaned for oxygen service. The lines are of ¼ in. o.d. 316 stainless steel tubing. The solution cell has telescoping windows permitting variable path length settings. This distance was calibrated prior to absorption measurements. All connections are 316 stainless steel Gyrolok fittings. *Warning! The authors advise anyone using elevated oxygen pressures above organic materials to proceed with extreme caution. In particular, the apparatus must be well-shielded and the oxygen pressure must never be increased rapidly. Also note the safety features in the pressure lines.* For the sake of the safety of anyone wishing to perform such experiments, we invite correspondence with regard to the high pressure apparatus and its operation.

Temperature control was achieved by circulating methanol, thermostated to the proper temperature, through cooling coils built into the cell and in direct contact with the solution cell. Dry Ice was used as a cold source for a 7-gal methanol reservoir from which the fluid was pumped through the cell. A small regain heater in this reservoir was employed on a duty cycle to prevent the alcohol from getting too cold. The bath was constantly agitated to ensure efficient mixing and no local temperature gradients throughout. A thermistor was used to monitor the temperature which never varied more than ±0.5°C for the duration of a typical 14-16 hr experiment. The temperature control system is illustrated schematically in Figure 2. All connecting tubing was well isolated with Armaflex foam insulation to prevent thermal loss to the environment. In any given experiment, the cell was prepared in an inert atmosphere box and charged with 50-100 psi of argon. The temperature was then allowed to equilibrate until no further changes were observed within a 2-hr period. The pressure of argon inside the cell was then measured using the gauge, the cell resealed at this recorded internal pressure, and the lines flushed with oxygen and reconnected to the cell. After an initial spectrum (to record A^0), oxygen was then admitted into the cell in excess of the internal presure to prevent back-flow of the argon into the lines. The oxygen pressure above solution for any one spectrum is therefore equal to $P_{gauge} - P_{argon}$. One-half hour was allowed for each pressure increment to equilibrate with the solution before a spectrum was recorded. The temperature of a solution was monitored constantly by a copper-constantan thermocouple in direct contact with the solution.

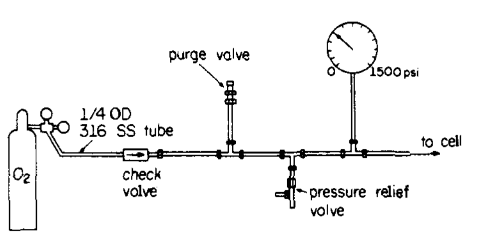

Figure 1. Schematic diagram of the gas handling system.

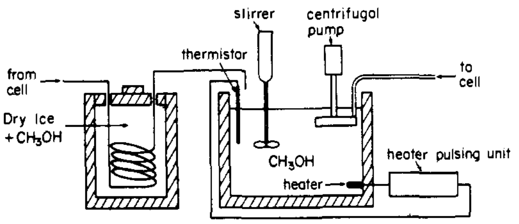

Figure 2. Schematic diagram of the temperature controlling system.

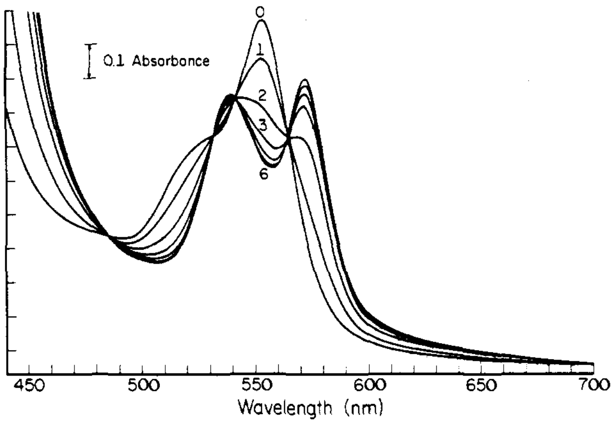

Figure 3. Spectral changes accompanying oxygen uptake at −41.6°C. Oxygen pressures of traces 1-6 are 0, 0.75, 3.54, 8.23, 12.59, 22.18, and 32.93 atm, respectively.

Results

On exposure to increasing pressures of oxygen, solutions of py-CoPIXDME exhibit changes in the visible spectrum characteristic of coordination of molecular oxygen. Each spectrum is retraceable independent of the direction from which this pressure is approached indicating complete re-

Table I. Spectrophotometric Data for Oxygen Uptake by Toluene Solutions of py·CoPIXDME

Run	p_{O_2}	Absorbance	Run	p_{O_2}	Absorbance
	$T = -5.9°C$ $b = 0.1277$ cm			$T = -41.6°C$ $b = 0.1277$ cm	
	$[py{\cdot}CoPIXDME]_0 = 4.33 \times 10^{-4} M$			$[py{\cdot}CoPIXDME]_0 = 4.26 \times 10^{-4} M$	
1	0	0.983	1	0	0.315
2	16.60	0.833	2	3.54	0.610
3	25.93	0.776	3	8.23	0.718
4	37.02	0.736	4	12.59	0.752
5	48.86	0.704	5	22.18	0.776
6	66.21	0.687	6	32.93	0.795
	$T = -29.6°C$ $b = 0.1294$ cm			$T = -52.7°C$ $b = 0.1294$ cm	
	$[py{\cdot}CoPIXDME]_0 = 4.06 \times 10^{-4} M$			$[py{\cdot}CoPIXDME]_0 = 3.66 \times 10^{-4} M$	
1	0	0.443	1	0	0.363
2	7.14	0.635	2	1.39	0.564
3	14.49	0.705	3	3.52	0.647
4	22.86	0.746	4	7.89	0.700
5	30.42	0.760	5	16.47	0.720
6	43.07	0.775	6	26.33	0.730

Table II. Calculated Equilibrium Constants and Deviations

Temp (°C)	K_{eq}	Marginal std dev	Conditional std dev	(Marg std dev)/(Cond std dev)
−5.9	0.0335	0.0044	0.0010	4.40
−29.6	0.138	0.0082	0.0033	4.03
−41.6	0.388	0.027	0.014	1.93
−52.7	0.753	0.034	0.022	1.55

versibility over the temperature range studied. Upon adduct formation, the single peak due to py·CoPIXDME at 555 nm decreases in intensity. At the same time, two new peaks at 540 and 573 nm appear. The spectra obtained at −41.6°C are shown in Figure 3. The experimental results are summarized in Table I. Spectra similar to those shown in Figure 3 were taken at three other temperatures. The procedure for interpreting these spectral changes and converting them to equilibrium constants and molar absorptivity differences has been described[3] in the literature along with some later modifications.[11] The results of this analysis are summarized in Table II, along with the marginal and conditional standard deviations and the ratio of marginal to conditional standard deviation. In all cases, this ratio is small. The absolute value of the conditional standard deviation is also small. All pressure measurements are taken assuming a standard state of 1 atm since, at the high pressures of these experiments, manipulation of the pressure in millimeters of mercury soon becomes unwieldy.

In none of the experiments was there any spectral indication of decomposition and/or oxidation of the metalloporphyrin moiety. This claim can be substantiated not only by the reproducibility of each trace upon returning to that particular pressure but also by the presence of four well-defined isosbestic points located at 486, 532, 542, and 555 nm. Had any decomposition to a third species occurred, it would have indeed been fortuitous for it to behave spectrally under the latter constraint.

The van't Hoff plot, shown in Figure 4, yielded from the slope an enthalpy of −7.80 kcal/mol and from the intercept an entropy of −48.7 eu calculated from a weighted linear regression analysis of the experimental points. Each point of the van't Hoff graph was given the weight $(K_i/\sigma_{K_i})^2$ appropriate to a plot of log K_i vs. $1/T_i$. This weighting scheme compensates the deviations for the application of the nonlinear natural logarithm operator to the experimental data. The error limits on the enthalpy and entropy are difficult to assess. Standard statistical techniques are not relevant to four points on a van't Hoff plot. Accordingly we report a standard deviation of 0.3 kcal mole^{-1} in ΔH and 1.3 eu in ΔS.

The assumption that oxygen behaves as an ideal gas is not valid under the elevated pressure used in these experiments. However, it must be stressed that the thermodynamic data for eq 1 represent a heterogeneous system and that all pressure readings were taken *after* the system had been allowed to come to equilibrium and are not initial readings. For this same reason, differences in the fugacities of the two gases always present in the cell are of no consequence in determining a thermodynamic equilibrium constant.

Discussion

Comparison of Thermodynamic Results. The enthalpy of interaction determined by us (−7.80 kcal mol^{-1}) differs significantly from that reported by Ibers et al.,[6a] who report an enthalpy of −9.2 ± 0.6 kcal/mol. The value previously reported for the entropy (−53 ± 3 eu) is within experimental error of ours (−48.7 eu).[12] This large negative entropy is consistent with the loss of the translational degrees of freedom of the oxygen molecule upon coordination. The magnitude of this number should be relatively insensitive to the particular system being investigated since the major contribution to it arises from the entropic loss of the free oxygen molecule. This statement is easily verified by reviewing previously reported entropies for oxygen coordination to various transition metal complexes.[5,13-16]

The enthalpy, however, is dependent on the electronic and steric properties of the transition metal complex. The rather low enthalpy determined in this experiment is consistent with the trend observed with increasing conjugation of the in-plane ligand. The difference of 1.4 kcal/mol between the value found in this work and that previously reported is a significant amount of the total interaction of 7.8 kcal/mol. In many systems, the maximum change in enthalpy that is observed over the entire range of base (or acid) variation is of the order of magnitude of the error in the data reported earlier[4] on this O_2 adduct. Moreover, the range of enthalpies previously reported for oxygen binding to various cobalt porphyrins as the base was varied only led to differences of 2.8 kcal mol^{-1} between the weakest and strongest system studied. The enthalpy of interaction of methylcobaloxime with a widely varying set of bases (N, O, and S donors) encompasses a range[17] of only 9 kcal mol^{-1}, and it was shown that errors of the order of magnitude of 1-3 kcal mol^{-1} masked many of the significant conclusions that could be drawn. It is precisely for this reason that we earlier labeled the O_2 binding systems as undefined. In a later section, we shall discuss sources of error in the limiting spectral approach.

Spectral Analyses. An important lesson can be learned from this example regarding the analysis of spectral data. The point is not, for example, should the error have been ±1.0 instead of ±0.4, but, instead, does the data provide a reliable definition of the system?

Possible outcomes of spectral data plotted using eq 3 or 4 are summarized in Figure 5. In each experiment, the computer will report a solution consistent in a least-squares sense with the experimental data. These plots can provide a wealth of chemical information that is lost in the computer output. Each line in the K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot refers to a different experimental trial (i.e., a different acid or base concentration, a different absorbance, or, in this case, a different pressure). An intersection of two lines corresponds to the solution of the two simultaneous equations for the two unknowns K^{-1} and $(\epsilon_C - \epsilon_A)$.

If the experiment were improperly designed, we shall show that these plots of the raw data would be invaluable in indicating why, and, therefore, provide information that could be used to correctly redesign the system.

When the experiment is properly designed and performed, a K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot approaching that in Figure 5a is obtained. Theoretically, for an exact determination of K^{-1} and $(\epsilon_C - \epsilon_A)$, the lines for the several experimental trials should all intersect at a single point. In practice, the intersections of the lines will form a small triangular region when the unknowns K^{-1} and $(\epsilon_C - \epsilon_A)$ are well determined due to errors inherent in the experiment. Furthermore, when K^{-1} and $(\epsilon_C - \epsilon_A)$ are determined, the several lines in the plot have greatly varying slopes.

When the slopes of all lines in the K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot are approximately the same (i.e., the lines of the plot are almost parallel as in Figure 5b), then there are many sets of K^{-1} and $(\epsilon_C - \epsilon_A)$ values which will serve almost as well as a solution to the series of simultaneous equations. This phenomena, subsequently[18,19] referred to as a saturation problem, was recognized in ref. 2b where several systems that suffered this shortcoming were reported. We can illustrate how the analyses by Person[18] and Deranleau[19a] are simply restatements of our parallel K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot statement. The slopes of the lines in the K^{-1} vs. $(\epsilon_C - \sigma_A)$ plot are obtained from eq 3 by differentiating K^{-1} with respect to $(\epsilon_C - \epsilon_A)$.

$$\frac{\partial(K^{-1})}{\partial(\epsilon_C - \epsilon_A)} = -\frac{A - A^0}{(\epsilon_C - \epsilon_A)^2} + \frac{C_BC_A}{A - A^0} \qquad (6)$$

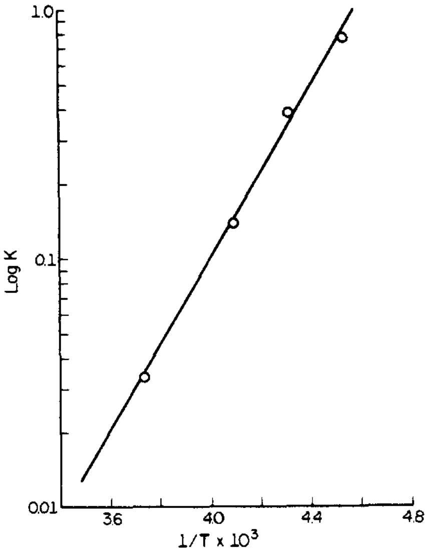

Figure 4. The van't Hoff plot for the data presented in Table II.

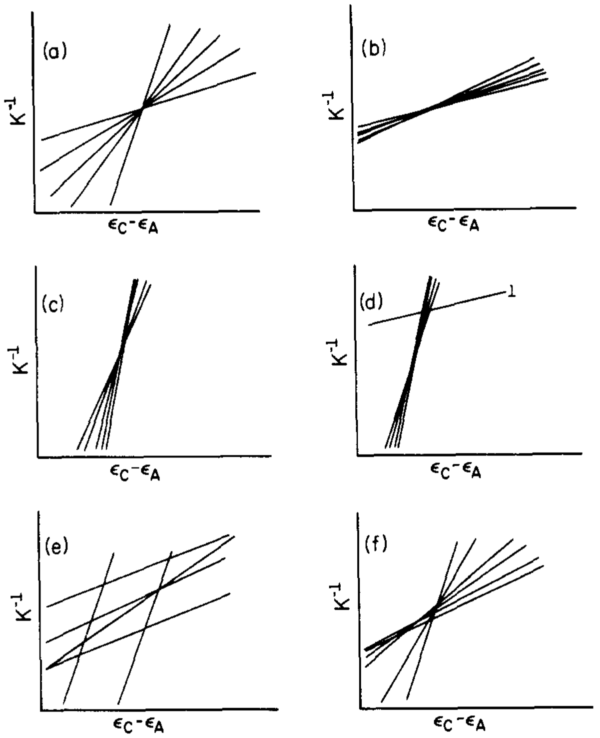

Figure 5. Illustrative plots of K^{-1} vs. $(\epsilon_C - \epsilon_A)$ which indicate various advantages of this representation.

The first term of eq 6 is negligible under conditions of a well-designed experiment and the second term is related to the quantity referred to as the saturation factor by Deranleau. If one selects $K = 50$, $C_A = 10^{-3}$ M and varies C_B from 2×10^{-3} to 0.2, it is a trivial matter to solve for the equilibrium concentrations and plot C_BC_A vs. C_C. Since C_C is related to $A - A^0$, one quickly sees Person's point that 60% of A must be complexed before the ratio $C_BC_A/(A - A^0)$ changes. If one assumes the acid to be the most dilute component in solution, this type of plot results from a small K and suggests that further experiments are needed in which more of the acid is converted to complex. The opposite condition results when a plot like Figure 5c is obtained. In all experimental trials, a very high percentage of the most dilute component was complexed but excess reagent was not employed; therefore, the range of saturation factors again is very limited. More experiments are needed in which less of the acid is complexed per increment of base addition. Thus, the phenomenon referred to as the "saturation problem" would hardly have been "overlooked by us",[4] for the problem was first described by us and was the main point of our criticism of the O_2 study. The Hill plot employed by Ibers et al. is described in the literature by Wyman.[20] Since our initial report in 1959, the shortcomings we discussed have been specifically demonstrated to exist in the Hill equation employed in the previous O_2 study.[4] According to Weber,[21] the reduction of spectroscopic data to any such linear form is to be particularly avoided.

A plot like that in Figure 5d illustrates a situation in which more experiments that vary slightly from the conditions used in experimental trial 1 (line 1) are needed to properly define the system. Line 1 of this plot corresponds to the most dilute solution employed where there is considerable error in the absorbance measurement. The intersection of this line with the other, nearly parallel lines determines the computed values of K^{-1} and $(\epsilon_C - \epsilon_A)$. Because

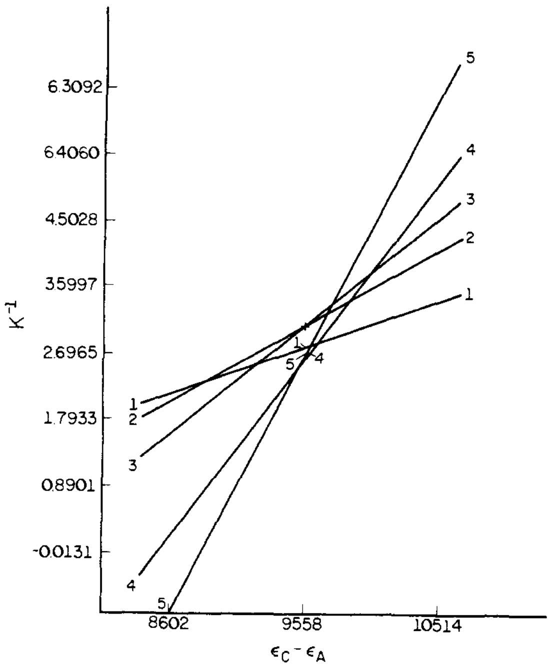

Figure 6. A typical K^{-1} vs. ($\epsilon_C - \epsilon_A$) plot for the data reported in this work.

line 1 contains considerable error, an unweighted least-squares analysis can produce a result in which there is considerably more error in the calculated results than indicated by the marginal standard deviation. For meaningful error limits, the random errors must be normally distributed. *This is hardly the case when there is only one line in the whole experiment with a slope different from all the others.* The nonexistence of a meaningful population distribution for different line slopes in the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots was the cause of our earlier rejection of the error limits reported on the O_2 system even though they were evolved "from a least-squares analysis". In summary, the reliability of statistics lies in the intelligence with which it is applied.

If a plot like Figure 5e is obtained, the system is not reproducible and the investigator should pay very close attention to his experimental procedure for parallel lines should fall directly on top one another.

There is one additional advantage of the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots which will be discussed. The trends in the values of K^{-1} for the intersections for various pairs of solutions can be examined to note whether or not a trend exists in the magnitude of the K intersections with increasing base concentration, e.g., Figure 5f. Such a trend is indicative of nonideal solution behavior (e.g., noncancellation of activity coefficient changes).

Thus, in all of these cases, the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots suggest modifications in the experimental procedure which lead to a better definition of the system. This is possible because the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots enable one to examine each experiment relative to the best fit rather than lumping all the experiments together in a summed squared error *which obscures the information* discussed above. Clearly, we have demonstrated that, contrary to other claims,[4] the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots are analytical aids which present a true display of the experimental data and contain a wealth of information. Figure 6 illustrates a typical K^{-1} vs. ($\epsilon_C - \epsilon_A$) plot for the data reported in this paper.

The Marginal and Conditional Standard Deviations. Recognizing that a tremendous amount of space would be required if each investigator had to publish K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots for every set of raw data that was collected in order to convince the reader of the quality of his work, we suggested that the ratio of the marginal standard deviation to the conditional standard deviation be reported in conjunction with the conditional standard deviation to summarize in relatively little space the appearance of his plot. We then proceeded to state some empirical results which relate values for these quantities to respectable plots. This clearly is an attempt on our part to introduce prejudices into the acceptable standards for this type of study. The results of the study reported here provide a very good reason for this prejudice. Introduction of prejudice is not foreign to a statistical analysis or to the interpretation of the resulting conclusions. The decision to weight or not to weight is an imposition of the investigator's prejudices as is the decision to define a certain R factor as acceptable.

If every single concentration range had so many experiments performed on it that each range of line slopes in the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots was statistically established, the graphical representation and our empirical description of it would not be required. However, most investigations are not carried out this way. The ratio used in conjunction with the conditional standard deviation is only a convenient, space-saving and relatively simple way to indicate the acceptability of the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots obtained. That is all it has ever been claimed[11] to be.

The ratio has physical significance. There is an error ellipse associated with the minimum detected in the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots. The ratio of the marginal standard deviation to the conditional standard deviation is simply an indication of the correlation in the two observables. As a matter of fact, in an elementary book on statistics,[22] this relationship is shown and an equation is given which relates this ratio to the correlation coefficient. A table relating the two quantities is provided from which for the example given a ratio of 10 *relates* to a correlation coefficient of 0.995. Thus, these rules do not constitute an "analysis that rapidly degenerates", nor do they "not represent the valid use of statistical quantities".[4] A plot like Figure 5a is described by a small marginal standard deviation *and* by a small conditional standard deviation, i.e., not only must the slopes of the lines of the plot vary greatly but they must all also intersect in a *small* triangular region. Because the direct visualization of this ratio (coupled with an acceptable value of the conditional deviation) in connection with the K^{-1} vs. ($\epsilon_C - \epsilon_A$) plots is simpler than the relation of the plots to the correlation coefficient, we prefer the ratio.

If the conditional standard deviation is 4–5% of the value of K (and if the experimental precision is greater), the results should be rejected for the error ellipse is very broad (see Figure 5e). Often a small ratio is obtained because the conditional standard deviation is large; however, these results are generally meaningless. If the precision of the measurement is only 4–5%, an independent determination of the stoichiometry is essential. If the conditional standard deviation is small (5% or less of the value of K), but the ratio is greater than 12, the calculated values of K^{-1} vs. ($\epsilon_C - \epsilon_A$) should be immediately rejected. This would correspond to an extreme case of Figure 5b or 5c where the error ellipse is narrow, yet quite elongated and the correlation coefficient close to 1. We label such systems as not defined for small systematic errors could have a drastic effect on the position of the minimum. A value of the ratio of three or less and a small conditional standard deviation indicates an acceptable K^{-1} vs. ($\epsilon_C - \epsilon_A$) plot (like Figure 5a) where the error ellipse approaches a small circle. This would imply that the slopes of the lines of the plot are dissimilar and intersect in a small triangular region and the correlation coefficient is 0.93 or less. If one finds a ratio between 3 and 12 and a small conditional deviation, he must examine the K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot to determine the extent of deviation of the plot from Figure 5a and thus the acceptability of the results. The plots for this case should be reported at least in the microfilm edition of the journal.

We shall next discuss a very important reason for insisting on plots like those in Figure 5a. In the case of the O_2 complexes studied, there is a wealth of data available to indicate that the complex is 1:1. However, in other systems in the solid state, the existence of such spectral data has not prevented investigators from carrying out an X-ray crystal structure determination—the results of which are then claimed to have proven the structure. In the absence of such a definitive tool in solution as X-ray crystallography, the obtaining of a K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot, like that in Figure 5a, is definitive proof of the stoichiometry of the adduct. In this connection, we thought it would be of interest to take data on a reported 2:1 complex and try to force it to fit a 1:1 expression. A range of base concentration was selected to correspond to the range of O_2 pressure variation used in the previous study.[5] The data utilized were those reported by Carlin and Losee[23] on the known 2:1 nickel dithiophosphate-4-picoline in benzene system. The least-squares procedure found a minimum at $K = 25.0$ l. mol^{-1} with a marginal deviation of 2.9 and a conditional deviation of 1.2 when the 2 to 1 system was forced to fit a 1:1 expression. This K and marginal deviation are obviously meaningless, and the system is not defined. Statistical analyses do have certain limitations. Certainly, the marginal deviation in this case indicates the system is better understood than it really is. In Figure 7, we have shown the K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot for the forced 1:1 fit of the 2:1 system. It is as good or better than most of the K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plots obtained on the reported O_2 binding data.[4]

Limiting Spectral Method. The limiting spectral analysis of spectrophotometric data used by Ibers et al.[4] contains many pitfalls. As shown by Vogel and Searby,[24] errors of approximately 20% are introduced by making this approximation in porphyrin systems where the base concentration only changes from 0.0148 to 0.132 M. This approximation has also been made in porphyrin systems[25] where the base concentration range is as low as 3.5×10^{-3} to 7.1×10^{-2} M eliminating any complication from a change in solvent properties with base concentration and errors on the order of 10% are reported to be introduced into the calculated value of K. The procedure of using a low temperature to fully form a complex and the use of this ϵ at a higher temperature is a highly questionable procedure. There is *no* reliable procedure for correcting the molar absorptivity for temperature. Complications in solution, e.g., varying extents of aggregation with temperature, make it difficult to make such corrections reliably. The influence of change in the $(\epsilon_C - \epsilon_A)$ value of iodine and the effect it has on the enthalpy of adducts has been reported in detail some time ago by Carlson and Drago.[26] It is also very difficult to distinguish between 95% complexation and 100% complexation. With a possible error in ϵ of 5-10% at $-70°C$ and with the possibility existing that this could change to any value inside the error limits at $-35°C$, a serious effect[25] on the error in $-\Delta H$ results. Thus, except in very favorable situations, we strongly advise against using limiting spectra in the analysis of data. Of course, *when* the molar absorptivity of all species can be directly and accurately measured at the temperature at which the experiment is being carried out, this procedure is recommended, for the accuracy in the resulting thermodynamic parameters is greatly enhanced when only one unknown (K) has to be determined. Unfortunately, the system shown in 5b is one where it is most difficult to get limiting spectra but is the system where a fair estimate of $(\epsilon_C - \epsilon_A)$ would be most valuable, for K is less dependent on the error. For Figure 5c, the limiting spectra can be most readily obtained, but slight errors have huge effects on the value of K.

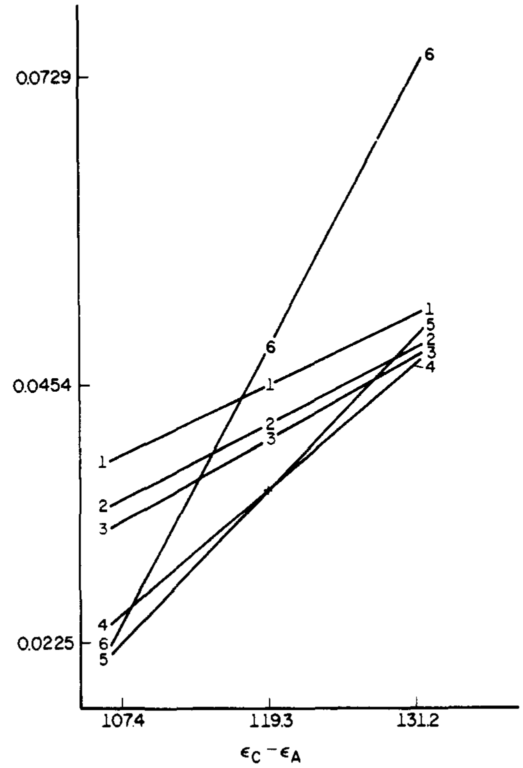

Figure 7. K^{-1} vs. $(\epsilon_C - \epsilon_A)$ plot from the interaction of nickel dithiophosphate with 4-picoline in benzene.

The effect of error in the $(\epsilon_C - \epsilon_A)$ from limiting spectra can also be seen from Figure 5. If, in Figure 5a, the limiting $(\epsilon_C - \epsilon_A)$ were 5% removed from the intersection, the values of K^{-1} for each individual run would be affected considerably and the average K would be quite different from that obtained by the simultaneous solution for K and $(\epsilon_C - \epsilon_A)$. This can be seen by drawing a vertical line on Figure 5a that is removed from the intersection. In Figure 5b, the effect would be minimal. Thus, by examining all the graphs, the general conclusion that can be drawn is that good data can be drastically affected by error in $(\epsilon_C - \epsilon_A)$ from a limiting spectra, but poor data (as illustrated in 5e) would not be. Furthermore, when K is large (the best conditions for getting $(\epsilon_C - \epsilon_A)$ from a limiting spectra), considerable error is introduced if the data are characteristic of those shown in Figure 5c.

The insistence by Ibers et al. on the advantages of their limiting spectra approach is indeed puzzling for the results from their own statistical analysis demonstrate it is inadequate (on their system). If the limiting spectral procedures were valid as claimed (for their system), the K from the limiting spectra would have to agree with and have smaller error limits than that determined by simultaneous solving for both K and $(\epsilon_C - \epsilon_A)$. Clearly, their reported value of 140 ± 9 from limiting spectra and a value of 86 ± 10 from the least-squares determination are seen to be inconsistent. If the assumptions of the limiting spectra were correct, the statistical analysis for K and $(\epsilon_C - \epsilon_A)$ tell us the K must be 86 ± 20 at 90% confidence. The value of 140 hardly qualifies.

Acknowledgment. We thank the National Science Foundation under Grant GP 31431X for support of this research. One of the authors (T.J.B.) would like to acknowledge fellowships from Standard Oil of California and the Chevron Research Company.

References and Notes

(1) R. G. Wilkens, *Adv. Chem. Ser.*, **No. 100**, 111 (1971), and references cited therein.

(2) (a) R. S. Drago, *Struct. Bonding* (*Berlin*), **15**, 73 (1973); (b) R. M. Guidry and R. S. Drago, *J. Am. Chem. Soc.*, **95**, 6645 (1973), and references therein.

(3) (a) N. J. Rose and R. S. Drago, *J. Am. Chem. Soc.*, **81**, 6138 (1959); (b) *ibid.*, **81**, 6142 (1959).

(4) J. A. Ibers, D. V. Stynes, H. C. Stynes, and B. R. James, *J. Am. Chem. Soc.*, **96**, 1358 (1974).

(5) H. C. Stynes and J. A. Ibers, *J. Am. Chem. Soc.*, **94**, 1559 (1972).

(6) (a) D. V. Stynes, H. C. Stynes, J. A. Ibers, and B. R. James, *J. Am. Chem. Soc.*, **95**, 1142 (1973); (b) D. V. Stynes, H. C. Stynes, B. R. James and J. A. Ibers, *ibid.*, **95**, 1796 (1973).

(7) One of the authors (T. J. B.) is indebted to the College of Agriculture, University of Illinois, for its generous donation of fresh beef blood.

(8) M. Grinstein, *J. Biol. Chem.*, **167**, 515 (1947).

(9) J. E. Falk, "Porphyrins and Metalloporphyrins", Elsevier, New York, N.Y., 1964, p 139.

(10) (a) J. R. Lakowicz, Ph.D. Thesis, University of Illinois, 1973; (b) J. R. Lakowicz and G. Weber, *Biochemistry*, **12**, 4161 (1973).

(11) F. L. Slejko, R. S. Drago, and D. G. Brown, *J. Am. Chem. Soc.*, **94**, 9210 (1972).

(12) Standard state is 1 Torr. To convert to a standard state of 1 atm the quantity (+*R* ln 760) must be added to this value.

(13) G. Amiconi, M. Brunori, E. Antonini, G. Tauzher, and G. Costa, *Nature* (*London*), **228**, 549 (1970).

(14) F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1154 (1973).

(15) A. L. Crumbliss and F. Basolo, *J. Am. Chem. Soc.*, **92**, 55 (1970).

(16) M. H. Keyes, M. Falley, and R. Lumry, *J. Am. Chem. Soc.*, **93**, 2035 (1971). These authors report an entropy of −60 eu/mol (standard state is 1 Torr) for oxygen uptake by sperm whale myoglobin.

(17) R. L. Courtright, R. S. Drago, J. A. Nusz, and M. S. Nozari, *Inorg. Chem.*, **12**, 2809 (1973).

(18) W. B. Person, *J. Am. Chem. Soc.*, **87**, 167 (1965).

(19) (a) D. A. Deranleau, *J. Am. Chem. Soc.*, **91**, 4044 (1969). (b) E. Kwiatkowski, *Z. Phys. Chem.* (*Leipzig*), **250**, 34 (1972).

(20) J. Wyman, *Adv. Protein Chem.*, **19**, 223 (1964).

(21) G. Weber and S. R. Anderson, *Biochemistry*, **4**, 1942 (1965).

(22) A. A. Clifford, "Multivariate Error Analysis", Applied Science Publishers, Ltd., London, 1973, pg. 26.

(23) R. L. Carlin and D. B. Losee, *Inorg. Chem.*, **9**, 2087 (1970).

(24) G. C. Vogel and L. A. Searby, *Inorg. Chem.*, **12**, 936 (1973).

(25) G. C. Vogel, private communication. We gratefully acknowledge Professor Vogel for helpful discussion on this point.

(26) R. L. Carlson and R. S. Drago, *J. Am. Chem. Soc.*, **85**, 505 (1963).

Steric Effects. V. Barriers to Internal Rotation

Marvin Charton* and Barbara Charton

Contribution from the Department of Chemistry, School of Science,
Pratt Institute, Brooklyn, New York, 11205. *Received February 19, 1975*

Abstract: Values of $\Delta G^{\ddagger}$ for the barriers to internal rotation in 11 sets of compounds were correlated with the modified Taft equation, $\Delta G^{\ddagger} = \psi\nu_{\mathrm{X}} + h$. With sets including more than four points, correlations were generally successful. The steric parameter (ν), is valid both for substituents bonded to aromatic rings and substituents bonded to aliphatic carbon atoms. The ψ values constitute a measure of the sensitivity to steric effects of a set of $\Delta G^{\ddagger}$ values for internal rotational barriers. The methyl group is found to behave as a symmetrical substituent with no noticeable "gear effect".

Recently, Nilsson et al.[1] have determined values of $\Delta G^{\ddagger}$ for barriers to internal rotation in substituted 1,3,5-trineopentylbenzenes (I). Attempts were then made to correlate these $\Delta G^{\ddagger}$ values with van der Waals volumes (V_{W}) and with $\Delta G^{\ddagger}$ values for rotational barriers in 2-substituted 2,3,3-trimethylbutanes[2] (II) by means of the linear free energy relationship

$$\Delta\Delta G^{\ddagger}_{\mathrm{IX}} = \gamma\Delta\Delta G^{\ddagger}_{\mathrm{IIX}} \qquad (1)$$

where $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{\mathrm{X}} - \Delta G^{\ddagger}_{\mathrm{H}}$. Good results were obtained with eq 1. An attempt to correlate $\Delta\Delta G^{\ddagger}$ values (with $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{\mathrm{X}} - \Delta G^{\ddagger}_{\mathrm{Me}}$) with the Taft[3] E°_{S} values obtained from the acid catalyzed hydrolysis of 2-substituted benzoate esters and esterification of 2-substituted benzoic acids was unsuccessful. It seemed of interest to us to examine the correlation of all available data on barriers to internal rotation as a function of substituent with the modified Taft equation

$$\Delta G^{\ddagger}_{\mathrm{X}} = \psi\nu_{\mathrm{X}} + h \qquad (2)$$

which is analogous to the equations used to correlate steric effects in previous papers of this series.[4-7] In eq 2, the ν values are steric parameters which are defined by the equation

$$\nu_{\mathrm{X}} \equiv r_{\mathrm{VX}} - r_{\mathrm{VH}} = r_{\mathrm{VX}} - 1.20 \qquad (3)$$

where r_{VX} and r_{VH} are the van der Waals radii of the X group and the hydrogen atom, respectively. The ν values are from ref 4 or from our compilation.[8] The value of ν used for the Ph group was 0.57. Correlations were carried out by the method of least-squares. Data used in the correlations are set forth in Table I. Results of the correlations with eq 3 are set forth in Table II.

Of the 11 sets of data studied, 4 gave excellent, 1 gave good, and 1 gave poor correlation. The remaining five sets did not give significant correlation. It should be noted, however, that four of these sets had only three points. Of the six sets with four or more points, five gave correlations ranging from excellent to good and only one did not give significant results. It seems likely therefore that the four three-point sets would have given much better results had more data been available.

Sets 3 and 7 both report $\Delta G^{\ddagger}$ values for barriers to internal rotation in 2-substituted 2-chloro-3,3-dimethylbutanes. The values obtained for the ethyl and *tert*-butyl groups in set 3 are in good agreement with those obtained in set 7, for which excellent correlation was obtained with eq 2. The values reported in set 3 for methyl and chloro agree fairly well with the value for methyl and chloro calculated from the correlation line for set 7. The value reported in set 3 for bromo is significantly larger than the value obtained from the correlation line for set 7. Furthermore in set 2, which reports $\Delta G^{\ddagger}$ values for internal rotational barriers in 2-substituted 2,3,3-trimethylbutanes, a system very similar to that studied in sets 3 and 7, the value of $\Delta G^{\ddagger}$ for the bromo substituent is only 0.3 kcal/mol greater than that of the