

On the Failure of the Guidry-Drago Reanalysis of Spectroscopic Data for the Reversible Oxygenation of Amine Complexes of Cobalt Protoporphyrin IX Dimethyl Ester

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Abstract: The reanalysis by Guidry and Drago of our spectroscopic data for the reversible oxygenation of amine complexes of cobalt protoporphyrin IX dimethyl ester is examined and shown to lack any redeeming features. A least-squares analysis is presented which supports our earlier analysis and which demonstrates the failure of the various empirical rules devised by Drago and coworkers for the assessment of spectroscopic data. Despite the general success of the least-squares approach used here for the simultaneous determination of the equilibrium constant and the molar absorptivity of the oxygenated species, arguments are presented in favor of our previous procedure of using limiting spectra in the derivation of the equilibrium constant.

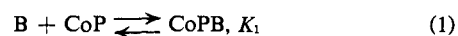
The binding of amine ligands as well as molecular oxygen to cobalt porphyrins in aprotic solvents has been the subject of several papers by us²⁻⁵ as well as by Walker.⁶⁻⁸ The fact that molecular oxygen will bond to CoPB, where P = porphyrin and B = base, enables one by spectroscopic means to determine equilibrium constants at various temperatures and from these the appropriate thermodynamic quantities. The thermodynamic quantities may be assessed for qualitative trends as a function of P or B and may also be compared with similar results for cobalt-substituted natural oxygen carriers.⁹ Comparisons between the natural systems and the synthetic systems enable one to assess the role of the globin in the binding of molecular oxygen to cobalt porphyrins.

In response to a request by Drago¹⁰ the raw data¹¹ used in the analysis²⁻⁵ were supplied. Guidry and Drago¹² (hereafter abbreviated GD) have now presented their reanalysis of our data. They conclude "... that, on the whole, the systems investigated by Ibers, *et al.*, were poorly defined and in most instances no numerical data can be obtained." In this paper we

first formulate the problem in some detail and then demonstrate that our earlier results are reliable. In so doing we demonstrate the inadequacy of the empirical rules devised by Drago and coworkers¹³ for the assessment of spectroscopic data.

Statement of the Problem

The following equilibria were studied by observing the changes in the spectra in the near-uv and visible regions.



Let ϵ_4 , ϵ_5 , and ϵ_6 be respectively the molar absorptivities of the four-coordinate (CoP), five-coordinate (CoPB), and six-coordinate (CoPBO₂) species. (In the notation of GD, $\epsilon_C = \epsilon_6$ and $\epsilon_A = \epsilon_5$.) Let q represent the total concentration of cobalt in solution, distributed over the four-, five-, and six-coordinate species. On the assumption that Beer's law holds and that B and solvent do not absorb in the region of interest, it is readily shown that the absorbance, A , of the solution as a function of the oxygen pressure, p , and base concentration [B] is given by

$$A = \frac{q\epsilon_5' + K_2pq\epsilon_6}{1 + (K_1[B])^{-1} + K_2p} \quad (3)$$

where

$$\epsilon_5' = \epsilon_5 + \epsilon_4/(K_1[B]) \quad (4)$$

Given A as a function of p , q , and [B] at a given temperature where the value of K_1 is available, then eq 3 in principle forms the basis for the determination of the unknowns K_2 , ϵ_5' , and ϵ_6 . If K_1 is large (so that $K_1[B] \gg 1$), a situation that obtains in our experiments, then eq 3 reduces to

$$A = \frac{q\epsilon_5 + K_2pq\epsilon_6}{1 + K_2p} \quad (5)$$

(13) Reference 12 contains sufficient bibliography to enable one to trace the evolution of Drago's ideas on this topic back to the initial paper of N. J. Rose and R. S. Drago, *J. Amer. Chem. Soc.*, **81**, 6138 (1959).

(1) (a) Northwestern University; (b) York University; (c) University of British Columbia.

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

(3) H. C. Stynes and J. A. Ibers, *J. Amer. Chem. Soc.*, **94**, 5125 (1972).

(4) D. V. Stynes, H. C. Stynes, J. A. Ibers, and B. R. James, *J. Amer. Chem. Soc.*, **95**, 1142 (1973).

(5) D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *J. Amer. Chem. Soc.*, **95**, 1796 (1973).

(6) F. A. Walker, *J. Amer. Chem. Soc.*, **92**, 4235 (1970).

(7) F. A. Walker, *J. Amer. Chem. Soc.*, **95**, 1150 (1973).

(8) F. A. Walker, *J. Amer. Chem. Soc.*, **95**, 1154 (1973).

(9) B. M. Hoffman, C. A. Spilburg, and D. H. Petering, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 343 (1971).

(10) These data were supplied in August of 1972, prior to the formal presentation of the thesis.¹¹ After Drago communicated to us the results of his 2 weeks' assessment of some 100 pages of raw data, there followed a period of correspondence in which both his analysis and our reanalysis (presented here) were discussed. From Jan 1973 on we were unaware of any further developments until Sept 13, 1973 when we received from Drago a preprint of his work¹² together with a copy of the computer program he used in his analysis.

(11) H. C. Stynes, Ph.D. Thesis, Northwestern University, 1972. The thesis contains an exhaustive tabulation of the raw spectroscopic data as well as an extended discussion of the experimental methods employed in its acquisition and the interpretive methods used.

(12) R. M. Guidry and R. S. Drago, *J. Amer. Chem. Soc.*, **95**, 6645 (1973).

Equation 5 can be recast into the following form

$$pK_2 = Y/(1 - Y) \quad (6)$$

where the fraction, Y , of total cobalt as CoPBO_2 is given by

$$Y = \frac{A_{\text{obsd}} - A_{\text{deoxy}}}{A_{\text{oxy}} - A_{\text{deoxy}}} \quad (7)$$

where A_{obsd} is the absorbance at a given p , A_{deoxy} is the absorbance of pure five-coordinate CoPB complex, and A_{oxy} is the absorbance of pure six-coordinate CoPBO_2 . A_{oxy} and A_{deoxy} are directly related to ϵ_6 and ϵ_5 , respectively. Y is identical with the saturation factor described by Person¹⁴ and by Deranleau.¹⁵ Equation 6 is a form of the Hill equation which GD indicate suffers from many pitfalls not inherent in their eq 3. Equations 5 and 6 and their eq 3 are simply different algebraic representations of the solution to the basic Beer's law equilibrium problem. As such they are identical, and none is inherently superior to another. If the data do not meet suitable saturation criteria,^{14,15} then there are pitfalls in the determination of K_2 from any of these equations.

Previous Analysis

As in any experimental science a thorough knowledge of the system at hand and its inherent limitations is a prerequisite to an intelligent interpretation of the data. We have previously considered in detail the problem of irreversible oxidation⁴ of CoPB and the question of the distribution of Co among CoP , CoPB , and CoPB_2 .^{5,11} The conditions of the oxygenation experiments were chosen so that irreversible oxygenation was minimized and so that typically greater than 95% of the Co is in the CoPB form before oxygenation. This distribution of Co was achieved with B concentrations in the range of 10^{-4} M and Co concentrations of about 10^{-6} M . It may be shown (see below) that the small amount of CoP present does not significantly affect the analysis. The much higher B concentrations necessary to produce 99+ % of the Co in the CoPB form would have introduced unnecessary complications because of solvent effects.³

The spectral data used for the evaluation of K_2 were obtained for given B and Co concentrations at temperatures between -30 and -65° for oxygen pressures between 0 and 760 Torr. The low temperatures are necessary in order to achieve appreciable oxygenation. Although with some bases still lower temperatures would have been desirable, the limitation of approximately -70° is imposed by the solvent, toluene, which becomes cloudy below this point. The low-temperature spectroscopic cell used in our experiments was limited to 760 Torr oxygen pressure. Moreover, the use of high oxygen pressures with organic solvents seems ill-advised. For a given base at least two sets of spectra were recorded at a given temperature. The spectral changes observed with increasing oxygen pressure included the occurrence of four isosbestic points at 564, 538, 528, and 485 nm which support the formation of CoPBO_2 in accordance with eq 2. Each spectral series was analyzed at two wavelengths, corresponding to the maximum absorbance of CoPB (≈ 552 nm) and the maximum absorbance of CoPBO_2 (≈ 573 nm). Con-

sequently for any given base there were invariably four, and usually more than four, independent determinations of K_2 .

It was clear from a cursory examination of the spectra that because of the limitations imposed by temperature and pressure it would be impossible with a few of the bases to obtain sets of data at the higher temperatures which would satisfy the saturation criteria.^{14,15} Since it is well known that attempts to determine K_2 and ϵ_6 simultaneously from data over a limited saturation range suffer from numerous pitfalls, regardless of the functional expression used, we decided on an independent measurement of ϵ_6 (or A_{oxy}) as a sensible means of analyzing the data. GD, while making reference to the saturation problem, have overlooked it in the re-evaluation of our data and have failed to make reference to the fact that the independent determination of ϵ_6 was used in our analysis.

Clearly ϵ_6 may be determined from a limiting spectrum¹⁶ from pure CoPBO_2 , but such a spectrum cannot always be obtained. A cursory inspection of the data of Table I, a repetition of Table II of ref 5, indicates

Table I. Thermodynamic Data for Reversible O_2 Binding to Cobalt(II) Protoporphyrin IX Dimethyl Ester · Ligand in Toluene Solution^a

Compound	Temp, °C	log K_{O_2} , mm ⁻¹	ΔH , ^{b,c} kcal/mol	ΔS , ^{b,c} eu
DMF · CoP	-31	-2.88 ^f		
	-45	-2.27	-11.0	-59
	-63.5	-1.34		
4-CNpy · CoP ^d	-45	-3.8		
	-63.5	-3.3		
py · CoP ^e	-45	-2.84		
	-57.5	-2.25	-9.2	-53
	-63.5	-2.09		
4- <i>t</i> -Bupy · CoP ^e	-37.4	-3.12		
	-45	-2.77	-9.8	-56
	-63.5	-1.97		
Im · CoP	-31	-2.50		
	-45	-1.84	-11.3	-58
	-57.5	-1.25		
CH ₃ Im · CoP ^e	-31	-2.36		
	-37.4	-2.04	-11.8	-59
	-45	-1.70		
4-NH ₂ py · CoP	-31	-2.58		
	-37.4	-2.34		
	-45	-2.05	-9.9	-53
pip · CoP	-63.5	-1.20		
	-31	-2.92		
	-45	-2.35	-9.0	-50
	-63.5	-1.65		

^a This is Table II of ref 5. ^b Standard state of 1 Torr. ^c The values of ΔH and ΔS were obtained from a weighted least-squares analysis of the data points. Since error estimates are not very meaningful for such a small number of data points, we have estimated the standard deviations derived from the least-squares analysis for all of the above data sets. We conclude that $\sigma(\Delta H) = 0.6$ kcal/mol and $\sigma(\Delta S) = 2$ eu and that errors within two standard deviations seem reasonable. ^d Thermodynamic data cannot be calculated with any accuracy due to large errors in K_{O_2} because of low oxygen affinity (<half oxygenated at -63.5° with 760 Torr O_2 pressure). ^e The values of ΔH and ΔS and their standard deviations differ slightly from those quoted in ref 2 and 4 because of the application of a more rigorous analysis in the present case. ^f Estimated errors in log K_{O_2} are ± 0.06 except for 4-CNpy where the errors are ± 0.3

(16) Because of the low concentration of O_2 in solution, this determination is not affected by the change in "donor concentration." (See, for example, G. C. Vogel and L. A. Searby, *Inorg. Chem.*, **12**, 936 (1973).)

(14) W. B. Person, *J. Amer. Chem. Soc.*, **87**, 167 (1965).

(15) D. A. Deranleau, *J. Amer. Chem. Soc.*, **91**, 4044 (1969).

that K_2 increases markedly as the temperature of the system is lowered and hence lowering the temperature is an effective way of driving the equilibrium of eq 2 to the right. Therefore we obtained values of ϵ_6 from solutions at $p = 760$ Torr at -70° . These values were extrapolated back to a given temperature by taking into account the solvent contraction effect and the change in vibronic structure.¹¹ For most of the bases examined the extrapolated values of ϵ_6 agreed closely with those derived on the assumption that limiting spectra had been achieved at the higher temperatures. For the bases py, *t*-Bupy, and especially 4-CNpy, this was not the case. This is evident from the $\log K_2$ values (Table I) obtained at the lowest temperatures for these bases. One can readily show that at $p = 760$ Torr the per cent saturation is 95, 89, 70, and 43% for $\log K_2$ values of -1.5 , -2.0 , -2.5 , and -3.0 mm^{-1} , respectively. Yet since the $\log K_2$ values for py and *t*-Bupy at -63.5° are around -2.0 mm^{-1} , it is very likely that greater than 90% of the Co is in the form of CoPBO_2 at -70° for these bases. Calculations for *t*-Bupy at $T = -37.4^\circ$ show that a 10% error in ϵ_6 results in about a 10% error in K_2 and hence to about a 2% error in $\log K_2$.¹⁷ For 4-CNpy it was impossible to obtain a reliable estimate of ϵ_6 and only approximate K_2 values are reported, and no thermodynamic quantities were derived.

Given the values of ϵ_6 (and hence A_{oxy}) eq 6 and 7 were employed in the derivation of K_2 values by a suitable plot of $Y/(1 - Y)$ vs. p . The four or more individual determinations of K_2 for a given base at a given temperature were invariably in good agreement with one another. Hence, these independent values of K_2 were averaged and generous error limits on the resultant values of $\log K_2$ of about 2–3% were assigned on the basis of our general feelings about the overall goodness of the fit and our knowledge of the experimental difficulties encountered. These $\log K_2$ values, together with the corresponding thermodynamic quantities derived from them, are given in Table I.

The GD Analysis

Our understanding of the methods employed by GD is limited to their paper,¹² the references cited therein, and to our analysis of the computer program supplied by them.¹⁰

At the outset we reiterate that our analysis was based on the premise that the value of ϵ_6 should be determined from limiting spectra, as described above, and that the single variable to be determined is K_2 . Basically GD employ a nonlinear, unweighted, least-squares analysis for the simultaneous determination of both K_2 and of $\epsilon_6 - \epsilon_5$, although since ϵ_5 is determined before addition of O_2 the practical variables in the GD analysis are K_2 and ϵ_6 . Following the solution for the best values (in a least-squares sense) of the variables, where the function minimized is

$$R = \sum_{\text{obsd}} (A_{\text{obsd}} - A_{\text{calcd}})^2 \quad (8)$$

the marginal and conditional standard deviations of

(17) Since $\sigma(\log K_2) = 0.43\sigma(K_2)/K_2$, for $\log K_2 = -2.0$ a 10% error in K_2 is reflected in approximately a 2% error in $\log K_2$. It is interesting that nowhere do GD point out this relationship even though it is the $\log K_2$ values that are used in the derivation of the thermodynamic quantities.

these variables are computed.¹⁸ A graphical analysis of K_2^{-1} vs. $\epsilon_6 - \epsilon_5$ is also made.

With respect to the mathematical procedures used by GD the use of unit weights is highly inadvisable. Otherwise, the analysis is a standard one, although the calculation of conditional standard deviations is unusual because such quantities have been little used in the literature of statistics. It is well known¹⁹ that the least-squares procedure, applied properly, assigns a weight to each observation that is inversely proportional to the variance of that observation. The assumption that all observations are measured with equal certainty is usually invalid. In the present instance examination of typical spectrophotometric data for these systems (refer, for example, to Figure 2 of ref 5) clearly indicates that at 552 nm the absorbance of curve 2 is known with far more certainty than is the absorbance of curve 8. Hence these observations should not be given the same weight, as GD have done. However, the unit weighting scheme is certainly to be preferred to the totally arbitrary one suggested earlier²⁰ but apparently not used in the present analysis.

When GD attempt to interpret their derived numbers, their analysis rapidly degenerates, as there follows a complete misunderstanding of the significance and use of the various statistical quantities involved, especially the marginal and conditional standard deviations.²¹ GD suggest *empirical* tests employing the marginal and conditional standard deviations. Typical of this empiricism is the statement by GD, "However, if the conditional standard deviation is small, but the ratio [of marginal to conditional standard deviation] is greater than 12, the values calculated for K and $\epsilon_C - \epsilon_A$ should also be immediately rejected. . . ." This does not represent the valid use of statistical quantities; rather it is an attempt on the part of GD to give credibility to their own prejudices by disguising such prejudices in the language of statistics.

Using a careful selection of our spectroscopic data and applying these remarkable testing procedures, GD reach the general conclusion that the values of K_2 , and hence the derived thermodynamic quantities, are meaningless.

Reanalysis of the Data

We have already indicated in some detail that we believe the proper analysis of the data in question pro-

(18) This calculation is made in a subroutine called "ERR." The version kindly sent to us by Drago would not work on a CDC 6400 computer using the FTN 3 compiler. When rewritten by us the subroutine gives marginal standard deviations that agree with our own least-squares analysis, if we use unit weights.

(19) See, for example, W. C. Hamilton, "Statistics in Physical Science," Ronald Press, New York, N. Y., 1964. A somewhat more mathematical account of the least-squares procedure is found in R. L. Plackett, "Regression Analysis," Oxford University Press, London, 1960.

(20) T. O. Maier and R. S. Drago, *Inorg. Chem.*, **11**, 1861 (1972).

(21) GD report marginal and conditional standard deviations to various confidence limits (see footnote *b* of Table I of ref 12). We presume that GD have not considered the interesting question of the standard deviation of the standard deviation but have simply misunderstood that there is a single, marginal standard deviation of a given variable and that this quantity, by appeal to an assumed population distribution, may then be used in assessing confidence limits for the variable itself. A statement by GD such as ". . . it is quite likely that the slopes of the curves are such that considerably more error exists in the calculated results than the marginal deviation indicates" support our contention that they do not understand the meaning of a marginal standard deviation as derived from the least-squares procedure.

Table II. Analysis of the B = MeIm Data at $T = -45.0^\circ$

Variables	Spectrum									
	OT 28 552 nm ^a		OT 28 572 nm ^b		OT 29 552 nm ^c		OT 29 572 nm ^d		Av ^e	
	log K_2^f	K_2^g	log K_2^f	K_2^g	log K_2^f	K_2^g	log K_2^f	K_2^g	log K_2^f	K_2^g
$K_2, \epsilon_6, \epsilon_5$	-1.74 (11) ^h	261 (68)	-1.70 (4)	283 (24)	-1.76 (6)	246 (34)	-1.73 (7)	275 (16)	-1.72 (3)	270 (18)
K_2, ϵ_6	-1.84 (9)	206 (42)	-1.71 (3)	277 (16)	-1.77 (5)	244 (26)	-1.76 (5)	250 (30)	-1.74 (2)	259 (13)
K_2, ϵ_6^i	-1.83 (9)	211 (43)	-1.70 (3)	284 (16)	-1.76 (5)	249 (26)	-1.75 (5)	256 (30)	-1.73 (2)	265 (14)
K_2	-1.77 (5)	242 (26)	-1.70 (1)	284 (9)	-1.71 (3)	275 (16)	-1.71 (3)	277 (19)	-1.70 (1)	281 (6)
K_2, ϵ_6 with unit weights	-1.78 (7)	235 (37)	-1.72 (3)	272 (16)	-1.74 (5)	259 (30)	-1.78 (5)	235 (27)	-1.74 (3)	258 (13)

^a Reference 11, p 248. ^b Reference 11, p 249. ^c Reference 11, p 250. ^d Reference 11, p 251. ^e Weighted average and standard deviation of the average computed on the assumption that individual values are from the same population. ^f Units of mm^{-1} , as used by us previously. ^g Units of l. mol^{-1} for comparison with the GD results. ^h The estimated standard deviation of the least significant figures is shown in parentheses here and in Table III. ⁱ The presence of the four-coordinate species was taken into account, using $\Delta H = -10.7 \text{ kcal mol}^{-1}$ and $\Delta S = 19 \text{ eu}$ to calculate K_1 for eq 1.⁵

ceeds from an independent determination of values of ϵ_6 and not from the simultaneous determination of ϵ_6 and K_2 , as advocated by GD. Nevertheless we undertook a reanalysis of the spectral data by least-squares procedures because our extensive experience with the method led us to be highly suspicious of the empirical criteria given by GD for assessment of spectroscopic data.

Returning to eq 3 it is a simple matter to write a nonlinear least-squares program that minimizes

$$WR = \sum_{\text{obsd}} w(A_{\text{obsd}} - A_{\text{calcd}})^2 \quad (9)$$

where the weight w is taken to be inversely proportional to the variance of A_{obsd} . In principle it is possible to determine any or all of the potential variables K_2 , ϵ_6 , and ϵ_5 that are involved in the calculation of A_{calcd} . The procedure is well known.¹⁹ For the multiwavelength problem at hand one could follow the elegant formulation of Lingane and Hugus,²² introducing a non-diagonal weight matrix, but, for the illustrative purposes we have in mind here, we have chosen the simpler course of separate evaluation of K_2 at each wavelength. The program was written so that any or all of K_2 , ϵ_6 , and ϵ_5 could be varied. Initial starting values for the parameters to be varied were usually derived assuming that we had observed limiting spectra. Least-squares cycles were continued until convergence was achieved; this usually required three or fewer cycles. The error analysis was a standard one with marginal standard deviations being derived from the inverse matrix and the error in an observation of unit weight. The correlation matrix was also examined.

Before presenting some of our results it is well to comment on one of the apparent problems in this type of analysis, a problem which GD were perhaps attempting to analyze empirically. Let us consider the two-variable problem, namely the determination of K_2 and ϵ_6 simultaneously from a given spectral data set. If a reasonable range of saturation is not achieved in the acquisition of this data set, then there will be a high correlation between K_2 and ϵ_6 . In principle, high correlation does not prevent convergence.²³ It appears not to be recognized by GD that such high correlation manifests itself quite naturally in large marginal stan-

dard deviations for the derived parameters. These standard deviations along with the correlation coefficients provide all the statistical quantities one needs to assess the solution. There is thus no utility or justification for the empirical rules of GD. Moreover, the plots of K_2^{-1} vs. $\epsilon_6 - \epsilon_5$, originally advocated by Drago and coworkers prior to their use of a fast computer and least-squares methods for this type of problem, should be replaced by a proper graphical representation, if it is felt that a visual aid is required. In this proper analysis one plots contours of WR (eq 9), the quantity being minimized, as a function of ϵ_6 and K_2 , the variables. The sharpness of the minimum of WR provides a visual display of the potential problems of solution for K_2 and ϵ_6 .

Although there is no place for empiricism in statistical analysis, the empiricism promoted by GD would be a harmless aberration were it not for the fact that it leads to invalid conclusions. This we now demonstrate.

We first present our reanalysis of the methyl imidazole (MeIm) data at -45.0 (Table II). These data have been chosen because although GD intimate that it is one of our better systems their graphical plot suggests to them that the results from these data may be meaningless. The results of Table II show that the GD interpretation of their graphical plots is meaningless.

Note, first of all, that our unweighted analysis of OT 28 552 nm leads to a value of K_2 of 235 (37) l. mol^{-1} (in the units used by GD). GD give the value for MeIm as 236 (34) l. mol^{-1} . These two values are in excellent agreement, considering the differences in computers and algebraic expressions. It is therefore blatantly clear that the statement in footnote 12 of ref 12 is untrue. GD did not do any averaging in the case of MeIm, but selected the *worst* of the four data sets given in ref 11.²⁴ We presume this was their standard procedure. Moreover, GD nowhere present any analysis, such as that in Table II, which indicates the general agreement among individual determinations of a given K_2 from different spectral runs.

Examination of the data of Table II leads to the following conclusions. (1) For MeIm letting ϵ_5 be an additional variable does not affect the results. (2) For MeIm allowance for the presence of the four-coordinate species has no significant effect on the derived quantities.

(24) This particular data set is poor because of an error in the value of A for the lowest nonzero oxygen pressure. The fact that this point is bad is immediately evident from the plot of $\log(Y/(1-Y))$ vs. $\log p$.

(22) P. J. Lingane and Z. Z. Hugus, Jr., *Inorg. Chem.*, **9**, 757 (1970).

(23) In practice the calculation may fail to converge depending upon the proximity of the correlation coefficient to unity and upon the word length of the computer. We demonstrate below that convergence was achieved in some instances when the correlation coefficient was greater than 0.98.

Table III. Reanalysis of *t*-Bupy Data

Variables	Minimum $ \rho(K_2, \epsilon_6) ^2$	$\log K_2^b$	Previous ^c $\log K_2$	K_2^d	$K_2(\text{GD})^e$
K_2, ϵ_6	0.85	-2.18 (5)	$T = -63.5^\circ$	86 (10)	"Not defined" (87 (23))
K_2		-2.07 (2)	-1.97 (6)	111 (6)	
K_2, ϵ_6	0.98	-2.69 (2)	$T = -45.0^\circ$	29 (1)	"Not defined" (28 (4))
K_2		-2.76 (2)	-2.77 (6)	30 (1)	
K_2, ϵ_6	?	osc ^f	$T = -37.4^\circ$		"Not defined"
K_2		-3.00 (2) ^g	-3.12 (6)	15 (1)	

^a Minimum absolute value of the correlation coefficient between K_2 and ϵ_6 . ^b Weighted average from four determinations at -63.5° and six determinations at -45.0° . Units are mm^{-1} . ^c Reference 5. ^d Units are l. mol^{-1} . ^e Reference 12. ^f Calculation would not converge on a CDC6400 with a 60-bit word length. Convergence may have been dependent upon starting parameters, but no mapping studies were made. ^g The four individual estimates of $\log K_2$ are -3.01 (3), -2.98 (3), -3.06 (4), and -2.95 (4) mm^{-1} , respectively. These values change by 2% with a 10% change in the assumed value of ϵ_6 .

This is a generally valid conclusion, borne out by similar tests on the other systems. Accordingly the concern of GD for the complications caused by the presence of CoP is unfounded. (3) The values of K_2 derived with ϵ_6 fixed or as a variable are the same. This supports the reliability of the ϵ_6 values derived for MeIm. (4) For any given model a simple statistical analysis of the four derived values of K_2 indicates that they are from the same population and hence that they may be averaged to yield the values and resultant errors in the last column of the table. The derived value for $\log K_2$ of -1.70 (1) mm^{-1} is in excellent agreement with that of -1.70 (6) mm^{-1} given earlier⁵ and indicates that our estimate of the error given previously is generous. (5) Even though the correlation coefficient between K_2 and ϵ_6 is only around 0.8, a relatively low value for these data sets, note that the resultant error in K_2 is nearly twice as great when K_2 and ϵ_6 are simultaneous variables as when K_2 is the single variable. The effects of correlation on the marginal standard deviations discussed above manifest themselves even under these rather mild conditions of correlation. (6) Regardless of the model chosen the calculation has converged to a well-defined minimum. Thus we conclude that the doubts raised in the GD analysis by reference to their handy plot of K_2^{-1} vs. $\epsilon_6 - \epsilon_5$ for this system have absolutely no validity. Their graphical plot, which is without statistical justification, is also without any practical merit in conjunction with a least-squares analysis.

We have carried out a reanalysis of our data for several of the bases considered by GD. Rather than present extensive new tabulations, tabulations which simply support our previous analysis, we consider one last example, namely that of *tert*-butylpyridine (*t*-Bupy). We chose this example because it presented some difficulties in the determination of ϵ_6 , as mentioned above, since with this base there is relatively poor binding of oxygen. We would thus expect it to represent the most challenging case for least-squares analysis.

We have considered two models for *t*-Bupy, namely the simultaneous determination of K_2 and ϵ_6 , and the determination of K_2 alone given our limiting values of ϵ_6 . The results are summarized in Table III. The following conclusions may be reached from these

data. (1) For the two lower-temperature sets the results from the two models agree, indicating that despite the difficulties discussed above our estimates of ϵ_6 are reliable. (2) Even though for the -45° data the absolute value of the *minimum* correlation coefficient between K_2 and ϵ_6 is 0.98, the calculation does converge to well-defined values. Again this points to the total failure of the GD empiricism with respect to analysis of spectroscopic data. (3) The clear superiority of our original method of analysis for these types of data is indicated by the results for $T = -37.4^\circ$, where the correlation coefficient between K_2 and ϵ_6 must be very nearly unity and where we did not achieve convergence. Yet use of our independently derived value for ϵ_6 leads to a reliable average value for K_2 and to excellent agreement among the four independent determinations of K_2 . (4) The values of K_2 derived from the present reanalysis fully support the values and error estimates given previously. The two sets of values do not differ significantly and do not lead to significantly different values for the thermodynamic quantities of interest.

Conclusions

In this paper we have demonstrated that the results of our previous analysis of the spectroscopic data in question are reliable. We have emphasized that for a variety of reasons we feel that the method of analysis used previously, based as it was on limiting spectra, is the preferred method. Nevertheless we have demonstrated by example that it is generally possible to derive K_2 and ϵ_6 simultaneously from our data by a nonlinear, least-squares method and that the results obtained agree with earlier results. In carrying out this least-squares analysis we have clearly demonstrated the failure of the Guidry-Drago¹² empirical rules for the assessment of spectroscopic data.

It is important to point out some additional mistaken impressions that may arise from the Guidry-Drago paper. Thus they state that a purpose of their reanalysis of our data was "... to ascertain whether a direct relationship does exist between the pK_a of the conjugate acid of the amine and the ΔH of formation of the Co-O bond in these systems ...". While results from our earlier work on a limited number of systems^{2,4} did indicate such a relationship, a cursory read-

ing of the completed work^{5,11} readily shows that the earlier relationship, based on limited data, was fortuitous. Our conclusion⁵ with respect to such a relationship is, "As with the binding of a ligand L to CoP, no simple, general relationship exists between ΔG , ΔH , or ΔS of the oxygenation of LCoP and the basicity of the ligand L."²⁵

GD have also intimated that there exist serious disagreements between Walker's⁶⁻⁸ results, based on esr studies of tetraphenylporphyrin and its derivatives, and our results,²⁻⁵ based on visible spectroscopic studies of protoporphyrin IX dimethyl ester. In fact, a careful reading of the papers in question will reveal that the

(25) The exact antithesis of this statement is presented by GD as one of our "essential conclusions."

only possible disagreement concerns the value of ΔH for MeIm relative to the other bases. While we differ on this point it is interesting that in terms of $-\Delta G$ the order found by Walker $py < pip < 4-NMe_2py < MeIm$ is identical with that found by us on a related but different system using a different technique.

Finally, while there are only small differences in the values of ΔH and ΔS for the various bases studied here, there are substantial differences between the results for these model systems and those for cobalt-substituted hemoglobin.⁹ These differences have proved to be important in the assessment of the role of the globin in the oxygenation of heme groups.

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Photochemical Reactions of the Azidopentaamminerhodium(III) Ion. Nitrene and Redox Reaction Paths

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Abstract: Photolysis of aqueous solutions of $Rh(NH_3)_5N_3^{2+}$ in 1.00 M HCl results in the evolution of nitrogen and the formation of $Rh(NH_3)_5NH_2Cl^{3+}$ and $Rh(NH_3)_5H_2O^{3+}$. The quantum yields are wavelength dependent, with the formation of $Rh(NH_3)_5NH_2Cl^{3+}$ favored at the longer wavelengths. The data have been interpreted in terms of the formation of coordinated nitrene and rhodium(II) intermediates. An intermediate whose spectrum is unlike that reported for flash photolysis is observed when $Rh(NH_3)_5N_3^{2+}$ is irradiated in glasses at 77° K. A model for the nitrene path is proposed in which the reactive level is a low energy azido group excited state populated by internal conversion or intersystems crossing.

Nitrene intermediates have been reported in the reactions of azide ion,^{1,2} hydrazoic acid,³ and of a large number of organic azides.⁴⁻⁶ However, reports of coordinated nitrene intermediates are conspicuously few.⁷⁻⁹ The formation of coordinated nitrenes in the reactions of $Ru(NH_3)_5N_3^{2+}$ and of $Ir(NH_3)_5N_3^{2+}$ with aqueous acid was established in reports from these laboratories.^{7,8} The chemistry of these intermediates was described as that of a strong electrophile, and they undergo a number of reactions which have analogs in both nitrene and organic nitrene chemistry.

In a recent communication from these laboratories, it was postulated that coordinated nitrene intermediates

are formed during the photolysis of $Ir(NH_3)_5N_3^{2+}$ and of $Rh(NH_3)_5N_3^{2+}$.¹⁰ Prior to this report the photochemistry of azido complexes had been interpreted in terms of substitution and/or azide radical formation with reduction of the central metal.¹¹⁻¹⁴ This paper reports a detailed study of the mechanism for the photodecomposition of the $Rh(NH_3)_5N_3^{2+}$ ion and the formation of $Rh(NH_3)_5NH_2Cl^{3+}$.

In a recent paper the mechanism for the photocomposition of $Ir(NH_3)_5N_3^{2+}$ was reported.¹⁵ In that report, a model for the photochemistry of coordinated azides was proposed. In that model it was proposed that a low energy excited state of the azido group was responsible for nitrene formation and that a charge transfer ligand to metal (CTTM) state of the azido complex was responsible for the photoreduction which has been observed for other azido complexes. The

(1) I. Burak and A. Treinin, *J. Amer. Chem. Soc.*, **87**, 4031 (1965).

(2) K. Glue, *Ber.*, **61**, 702 (1928).

(3) A. O. Beckman and R. G. Dickinson, *J. Amer. Chem. Soc.*, **50**, 1870 (1928).

(4) W. Lworski and T. Mattingly, *J. Amer. Chem. Soc.*, **87**, 1947 (1965).

(5) G. Smolinsky, E. Wasserman, and W. A. Yager, *J. Amer. Chem. Soc.*, **84**, 3220 (1962).

(6) (a) A. Reiser, G. Bowes, and R. J. Horne, *Discuss. Faraday Soc.*, **62**, 3162 (1966). (b) A. Reiser and T. Willets, *Nature (London)*, **211**, 4101 (1966).

(7) L. A. P. Kane-McGuire, P. S. Sheridan, F. Basolo, and R. G. Pearson, *J. Amer. Chem. Soc.*, **92**, 5865 (1970).

(8) B. C. Lane, J. W. McDonald, F. Basolo, and R. G. Pearson, *J. Amer. Chem. Soc.*, **94**, 3786 (1972).

(9) H. Kwart and A. A. Kahn, *J. Amer. Chem. Soc.*, **89**, 1950 (1967).

(10) J. L. Reed, F. Wang, and F. Basolo, *J. Amer. Chem. Soc.*, **94**, 7172 (1972).

(11) W. Beck and K. Schorpp, *Angew. Chem., Int. Ed. Engl.*, **9**, 735 (1970).

(12) C. Bartocci and F. Scandola, *Chem. Commun.*, 531 (1970).

(13) A. Vogler, *J. Amer. Chem. Soc.*, **93**, 5912 (1971).

(14) J. F. Endicott, M. Z. Hoffman, and L. S. Beres, *J. Phys. Chem.*, **74**, 1021 (1970).

(15) H. D. Gafney, J. L. Reed, and F. Basolo, *J. Amer. Chem. Soc.*, **95**, 7998 (1973).