# Polarization Characteristics of Flavin Spectra. Specular Reflectivity of Bis(10-methylisoalloxazine)copper(II) Perchlorate Tetrahydrate[1a]

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**Abstract:** Specular reflection spectra have been measured in the 212–660 nm region for the (100), (001), and (10$\bar{2}$) faces of crystalline bis(10-methylisoalloxazine)copper(II) perchlorate tetrahydrate and have been converted to absorption spectra by applying the Kramers–Kronig transformation. The two low-energy bands, nominally occurring at 450 and 370 nm in riboflavin, appear to be single transitions. Their transition dipole directions are given by $\theta$ = 26 and 1°, respectively, in a reference system where $\theta$ is measured clockwise from the N(10)–N(1) direction. Two peaks, probably vibrational, appear within the first transition, at 19.7 and 21.0 kK. The third, nominally 280 nm, solution band contains at least two strong transitions. The first of these occurs at $\theta$ = 3° and the second at $\theta$ = −19°. A fifth transition with $\theta$ approximately 42° comprises the band lying near the observational limit in aqueous solution at ca. 220 nm. Peak energies and isotropic oscillator strengths for the five transitions are 19.7, 25.3, 35.2, 38.9, and 45.3 kK, and 0.16, 0.15, 0.20, 0.39, and ca. 0.11, respectively. The 25° angle between transitions I and II generally agrees with earlier measurements.

Riboflavin is a versatile biochemical catalyst[2] which participates in reactions that are significant primarily because of their electron transport function and also in reactions where the chief significance is biosynthesis or catabolism. Its coreactants include aromatic and nonaromatic materials and such metal-containing groups as cytochromes, iron–sulfur clusters, and possibly molybdenum-containing centers. It may also serve as a photoreceptor.

Since an understanding of catalytic properties requires accurate calculation of electronic states, we are collecting data which will help evaluate different calculational approaches for predicting properties of the flavins. One type of such critical but sparsely available information consists of the directions of the electronic transition moments.[3] When known, these directional characteristics, or polarizations can also be used to estimate the orientation of the chromophore in crystals and other ordered systems.[3,4] Until now,[5] experimental polarization studies on flavins have been limited to the determination of the angle between the first (450 nm) and second (370 nm) transition moments by polarized fluorescence studies of glasses and stretched films.[6,7,9] The absolute orientations have been estimated by various molecular orbital treatments.[6,10–13]

Measurements of absolute transition moment directions require a sample in which the molecular orientation is known, such as a single crystal. However, crystals containing highly absorbing species such as the flavins are at best difficult, and often impossible, to study in direct absorption. The present study has thus employed the techniques of specular (or mirrorlike) reflection where, generally speaking, the reflectivity increases as the absorption becomes more intense. The resulting reflection spectra have been transformed via the Kramers–Kronig procedure to yield absorption spectra that correspond to those that would have been obtained in direct measurement.[14]

The riboflavin crystal structure has not been determined and crystals of the simplest electronically similar material, 10-methylisoalloxazine (I), are of very irregular habit[15] and thus unsuitable for measurements of the kind described here. However, complexes of 10-methylisoalloxazine with various metal salts have been characterized crystallographically. The monoclinic cupric perchlorate complex[16] was chosen for study because of its favorable crystal symmetry and habit.



I
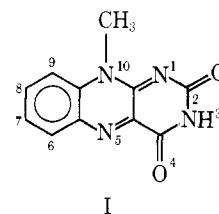
## Experimental Section

Well-formed orange-brown crystals of bis(10-methylisoalloxazine)copper(II) perchlorate tetrahydrate were grown by slowly cooling a mixture prepared by adding hot nearly saturated aqueous cupric perchlorate to a concentrated solution of 10-methylisoalloxazine in formic acid. A mixture of habits was observed, but invariably many of the crystals displayed the roughly tabular form shown in Figure 1, in which faces of the [010] zone were well developed and easily indexed. In these faces, the principal or extinction directions are constrained by symmetry to lie parallel and perpendicular to b and one need therefore not be concerned about dispersion (or variation with wavelength) of the principal direction. The (100), (001), and (10$\bar{2}$) faces were studied in the present investigation. They were initially identified by x-ray photography and later by optical goniometry; the angle between (001) and (100) is 69° and the (00$\bar{1}$), (10$\bar{2}$) angle is 52°.

The microspectrophotometer used in measuring the specular reflectivities has been described.[14,17,18] It consists of a normal incidence Leitz microscope equipped with quartz or reflecting optical elements and a Glan polarizing prism, a Bausch and Lomb 250 mm grating monochromator, and an RCA 1P28 photomultiplier detector. A tungsten projection lamp and a deuterium arc lamp were used as light sources, and a chopper in the incident beam coupled with phase sensitive detection eliminated the need for separate measurement of stray light.

A typical run consisted of aligning the crystal face normal to the instrument's optical axis with one of the two crystal extinction directions in that face parallel to the electric vector of the polarized incident radiation, measuring the reflectance spectrum in a point by point fashion, then rotating the crystal through 90°, checking its alignment, measuring the second spectrum, and finally replacing the crystal with an accurately aligned aluminum mirror and measuring the mirror reflectivity. During this group of three spectra all experimental parameters except crystal and mirror alignment were untouched. Intensity drift from beginning to end of a run was checked on occasion and found to be less than 1–2%. The photomultiplier
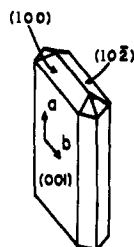
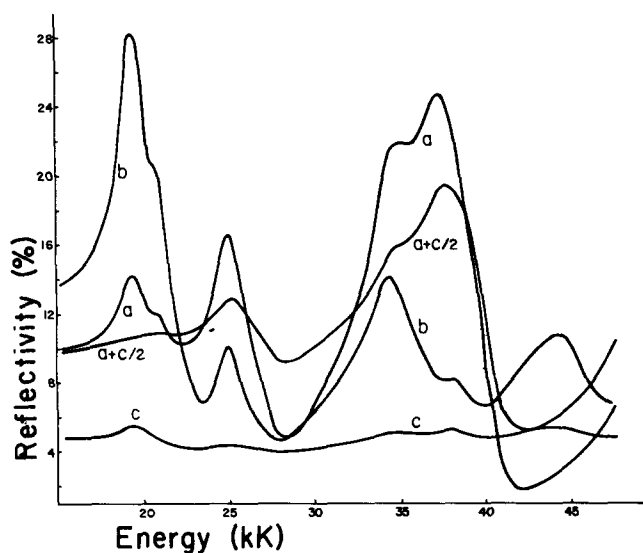**Figure 1.** Crystal habit of bis(10-methylisoalloxazine)copper(II) perchlorate tetrahydrate.



**Figure 2.** Crystal reflectivities. The label indicates the crystal direction parallel to which the incident radiation was polarized.

read-out was kept within 20–80% of its full scale range whenever possible by a combination of stepped sensitivity settings and uniformly shading screen filters. Any change in filter or sensitivity was accompanied by intensity readings made before and after the change to establish the new scale.

Division of crystal reflectivity by mirror reflectivity at each wavelength gave a relative crystal reflectivity, which was made absolute by multiplying by the previously determined absolute mirror reflectivity for that wavelength. Spectra were measured at every 2 nm from 212 to 300 nm, every 3 nm from 300 to 360 nm, every 5 nm from 360 to 400 nm, and every 10 nm from 400 to 660 nm. A resolution of 6.6 nm in illumination was maintained throughout. In one experiment, to confirm absence of significant absorption beyond 660 nm, measurement on the (001) face was extended to 1200 nm using an RCA 7102 detector cooled to −20 °C.

The reflectivities displayed in Figure 2 are the averages of the best three runs on each crystal face, except for the **b** spectrum, which is the average of spectra measured on both (001) and (100). Each spectrum was measured on a minimum of two crystals. The principal criterion used in choosing the best spectra was that of maximum reflectivity, since mirror alignment was relatively easy and all reasonably expected crystal errors or surface imperfections lead to lower reflectivity. Reflectivities of spectra accepted lay within ±5% of the average.

The uv–visible spectrum of 10-methylisoalloxazine in water was measured on a Cary 14 recording spectrophotometer. The infrared spectrum of 10-methylisoalloxazine in KBr was measured on a Beckman IR8.

### Data Analysis

The absorption spectra shown in Figure 3 were obtained from the measured reflectivities through a Kramers–Kronig analysis[14,17–19] using approximations outlined previously.[14] The reflectivity at all energies below the lowest energy measured was considered to be equal to the reflectivity of that point, and the effects of reflectivity in the experimentally inaccessible
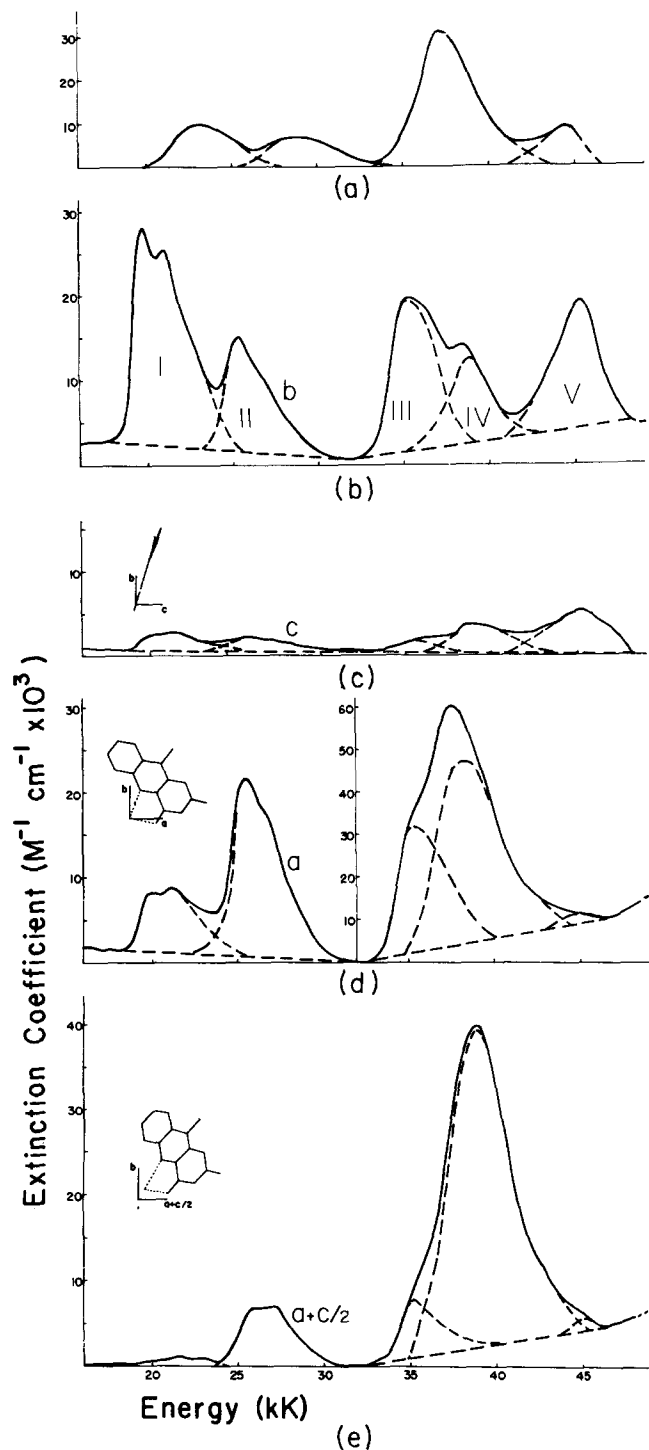


**Figure 3.** Solution and derived crystal absorption spectra, with proposed resolution into components. The solution and the crystal spectra are those appropriate for randomly oriented and for ordered absorbing species, respectively. The crystal molarity used in the Kramers–Kronig analysis was 4.50. Inserts show one molecule projected onto each crystal face, with dotted lines indicating the coordinated copper(II) ion. (a) Aqueous solution of 10-methylisoalloxazine; (b) average **b** spectrum; (c) **c** spectrum, (100) projection; (d) **a** spectrum, (001) projection; (e) **a** + **c**/2 spectrum, (10$\bar{2}$) projection. Note the changes in vertical scale

vacuum ultraviolet were approximated by an "effective transition"[14] added in that region The reflectivity above the terminus of this effective transition was held constant at the last value given. The strength and position of the effective transition were adjusted to avoid negative extinction coefficients ($\epsilon$) and, so far as possible, to minimize the tendency toward a generally rising background in the higher energy region. Because all
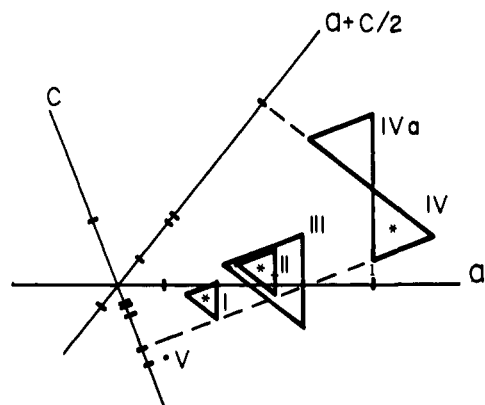
**Figure 4.** Consistency of projected moments. Only those moment projec-
tions which lead to the smallest error triangles are shown. The method of
constructing these triangles is illustrated for transition IV. Alternate tri-
angles IV and IVa are discussed in the text.

**Table I.** Integrated Absorption Bands

|     | $\nu^a$ | $A_{M,a}{}^b$ | $A_{M,b}{}^b$ | $A_{M,c}{}^b$ | $A_{M,(a+c/2)}{}^b$ |
|-----|------|-------|------|------|-------|
| I   | 19.7 | 29.2  | 88.9 | 7.6  | 3.6   |
| II  | 25.3 | 74.6  | 40.6 | 4.7  | 25.4  |
| III | 35.2 | 104.7 | 57.5 | 4.0  | 20.3  |
| IV  | 38.9 | 199.3 | 31.6 | 14.3 | 167.2 |
| V   | 45.3 | 6.4   | 49.7 | 21.9 | 2.2   |

$^a$ kK. $^b$ $M^{-1}$ cm$^{-2}$ × $10^6$.

**Table II.** Oscillator Strengths and Possible Moment
Orientations$^a$

| Transi- |     | M·b > 0 |        | M·b < 0 |        |
| tion    | $f$ | $\chi$, deg | $\theta$, deg | $\chi$, deg | $\theta$, deg |
|---------|------|------|------|------|------|
| I   | 0.16 | −27 | 76  | 2   | 26  |
| II  | 0.15 | −20 | −78 | 0   | 1   |
| III | 0.20 | −22 | −79 | −1  | 3   |
| IV  | 0.39 | −13 | −58 | −2  | −19 |
| V   | 0.11 | −49 | 60  | −18 | 42  |

$^a$ $\chi$ is the angle between M and the molecular plane. $\theta$ is defined
in the text.[21]

spectra measured with the polarization vector parallel to a
given crystal direction should be identical, a **b** spectrum based
on the average of the reflectivities given by faces (001) and
(100) was used and is shown in Figure 3. Since the extremely
small (10$\bar{2}$) faces required higher magnification, the spectra
measured on this face were considered less reliable.

The correspondence of bands in the crystal absorption
spectra with those in the solution spectrum of 10-methyl-
isoalloxazine, coupled with the agreement between the solution
and the crystal intensities that are ultimately obtained, suggests
that an "oriented-gas" interpretation of the crystal absorption
spectra may be appropriate. Strictly speaking, in such a model
one ignores interactions between molecules and considers each
of the measured spectra to result from interaction of a polarized
incident light beam with a collection of molecules having the
orientations found in the crystal. In such a case, for an elec-
tronic transition having transition moment **M** and an oriented
vapor phase extinction coefficient $\epsilon(\nu)$ at any wavenumber $\nu$,
the fraction of $\epsilon$ observed in any particular spectrum is $\epsilon_{M,k}(\nu)$
$= \epsilon(\nu) \cos^2 \varphi_{M,k}$ where **k** is the direction of measurement and
$\varphi_{M,k}$ is the angle between **M** and **k**.

The assumptions of the analysis employed here are not as
stringent as in an oriented gas analysis, however, and require
only that the crystal interactions that do occur be such that the
model still applies to the transition moment directions. For a
crystal of the space group symmetry $P2_1/c$ found here, four
orientationally distinct molecules occur, but all four have
identical $\cos^2 \varphi_{M,k}$ for all **M** and any **k** parallel or perpendicular
to **b**. For the purposes of calculating intensity, attention may
thus be focussed on one molecule.

If one considers the direction of **M** to remain nearly constant
across an electronic band it is apparent that a minimum of five
transitions are required to explain the four principal regions
of absorption. That is, although the transitions centered near
19.7, 25.3, and 45.3 kK can be considered single, two transi-
tions centered near 35.2 and 38.9 kK are required to explain
the variations found in this region in the four spectra.[20] These
transitions will be denoted I–V in order of increasing energy.
Figure 3 shows an average among the extreme possibilities for
resolving the generally overlapped bands that are associated
with transitions I–V. If one integrates the area under each
resolved band of Figure 3, a quantity $A_{M,k}$ may be calculated
that is simply related to the molecular oscillator strength:

$$A_{M,k} = \int \epsilon_{M,k}(\nu) \, d\nu = 6.94 \times 10^8 f_M \cos^2 \varphi_{M,k} \quad (1)$$

where $f_M$ is the (isotropic) oscillator strength of the transition
having moment **M**. Values of $A_{M,k}$ are given in Table I.

At least three polarized spectra are required for calculation

of moment directions; even then fourfold ambiguity remains.
The presence of four spectra permits reduction to twofold
ambiguity and as the following analysis shows, the symme-
try-imposed restriction that transitions in planar systems are
electronically allowed only parallel or perpendicular to the
molecular plane permits confident assignment of unique di-
rections to all moments.

The construction used in determining moment directions
is as follows. Because $f$ for a given band is proportional to the
square of the corresponding transition dipole moment **M**, the
observed quantities $(A_{M,k})^{1/2}$ are proportional to projections
of **M** onto each direction of measurement **k**. The terminus of
**M**, suitably normalized, must thus fall on one of a pair of planes
normal to **k** and at a distance $\pm(A_{M,k})^{1/2}$ from the origin.
Three nonzonal pairs of these planes yield a parallelepiped
whose four unique corners are the permitted values of **M**. In
the present case, the three zonal vectors **a**, **c**, and (**a** + **c**/2)
yield planes whose traces can conveniently be shown in the **a**,**c**
plane (Figure 4) and which limit the permitted **M** values to two
for each band. The projection of **M** onto **a** is arbitrarily as-
sumed positive. It can be seen that experimental or resolution
error converts the triple planar intersections which ideally are
points into the "error triangles" shown. The center of the
smallest such triangle is chosen in each case as the desired
projection of **M** onto the **a**,**c** plane. The only ambiguity is as-
sociated with transition IV, for which two nearly equal trian-
gles, IV and IVa, result. The requirement that the sum of areas
of bands III and IV be constant and therefore that resolution
errors be of opposite sign leads to elimination of IVa.

Combining the unique projection of each **M** from Figure 4
with the two permitted **b** projections leads to the results in
Table II.

## Results and Discussion

**Moment Directions and Oscillator Strengths.** One of the two
possible directions of **M** for each transition given in Table II
lies within 18° of the ligand plane,[21] and only for band V is the
angle larger than 2°. This fact and the agreement of crystal
and solution oscillator strengths (Table III) provide convincing
evidence both that the five transitions are $\pi$–$\pi^*$ in nature, and

Table III. Observed and Calculated Transition Moments in 10-Methylisoalloxazine and Related Systems

| Transition | Obsd | | | | | | Calcd | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cu crystal[a] | | | H2O soln[a] | | A[b,c] | | B[b,d] | | | C[e] | | | D[f] | | |
| | $E^g$ | $f^h$ | $\theta$, deg | $E^g$ | $f$ | $E^g$ | $f$ | $E^g$ | $f$ | $\theta$, deg | $E^g$ | $f$ | $\theta$, deg | $E^g$ | $f$ | $\theta$, deg |
| I | 19.7 21.0 | 0.16 | 26 | 23.1 | 0.18 | 24.7 | 0.56 | 25.8 | 0.57 | 5 | 22.5 | 0.65 | 9 | 22.6 | 0.69 | 10 |
| II | 25.3 | 0.15 | 1 | 28.8 | 0.14 | 30.9 | 0.12 | 32.4 | 0.17 | −37 | 28.0 | 0.14 | −25 | 29.0 | 0.12 | −10 |
| | | | | | | 32.8 | 0.06 | 33.5 | 0.07 | −89 | 34.8 | 0.04 | −73 | 33.6 | 0.20 | −28 |
| III | 35.2 | 0.20 | 3 | 37.2 | 0.55 | 37.5 | 0.14 | 40.0 | 0.23 | | 36.5 | 0.59 | −37 | 35.7 | 0.60 | −49 |
| IV | 38.9 | 0.39 | −19 | | | 36.4 | 0.94 | 40.1 | 0.85 | | | | | 37.7 | 0.07 | |
| V | 45.3 | ∼0.11 | ∼42 | 44.5 | ∼0.11 | 41.6 | 0.17 | | | | | | | | | |
| | | | | | | 45.1 | 0.02 | | | | | | | | | |
| | | | | | | 46.4 | 0.63 | | | | | | | | | |

[a] 10-Methylisoalloxazine; this work. [b] Isoalloxazine. [c] Reference 10. [d] Reference 12. [e] 7,8-Dimethylisoalloxazine; ref 11. [f] 7,8,10-Trimethylisoalloxazine (lumiflavin); Figure 1, ref 6. [g] kK. [h] Isotropic oscillator strength (eq 1), for direct comparison with solution measurement.

that the oriented gas approximation is reasonable. If one assumes in each case that the orientation with smaller $|\chi|$ is correct, then the five transitions are located at $\theta = 26, 1, 3, -19$, and ca. 42°, respectively. These most probable directions are illustrated in Figure 5. One must add the caveat that transition IV could possibly occur at the alternate value of 58°, corresponding with a somewhat greater apparent out-of-plane angle of 13°.

An estimated standard deviation of 3–4° or a maximum estimated error of ca. 10° can be associated with bands I–IV. Error limits are perhaps twice as large for band V, the band most severely affected by approximations in the Kramers-Kronig analysis and by possible overlap with transitions outside the region of measurement. These estimates are subjective, but are supported by the small values found for $|\chi|$.[24] Oscillator strengths have estimated standard deviations of ca. 6%, except for transition V, where $f$ should be considered an order of magnitude estimate.

Well-resolved structure appears in transition I, and partially resolved structure in transition II. In both cases, separations are near 1300 cm⁻¹. These are assumed to be vibrational in nature, possibly related to the numerous bands in the region 1180–1700 cm⁻¹ or to a sharp 640-cm⁻¹ band in the ligand's infrared spectrum. Eaton et al.[5] record a similar separation of ca. 1000 cm⁻¹ between the less well resolved components of transitions I and II in the riboflavin-5′-phosphate cofactor of *Clostridial* flavodoxin.

**Possible n–π\* Transitions.** Fortuitously, the molecular plane is nearly perpendicular to (100), as Figure 3 illustrates, and any n–π\* transitions should appear with maximum intensity in the c spectrum. No significant unexplained intensity is present; any n–π\* transitions in the energy range observed must thus be weak. A conservative upper limit of $\epsilon$(isotropic) ≤ 1000 M⁻¹ cm⁻¹ can be estimated for any such transitions.

## Correlation with Other Data and Calculations

**Experimental Data.** Sun, Moore, and Song,[6] Lhoste,[7] and Siodmiak and Frackowiak[9] agree on the basis of polarized fluorescence spectra of various flavins in glasses and stretched films that each of the 450- and 370-nm bands contains a single strong transition, and that the angle between transition moments I and II lies in the range 9–35°. These values are in good agreement with our measurement of 25°. Eaton et al.[5] report $\theta = 16°$ for band I and $\theta = -4°$ for band II.[25] Their $\Delta\theta_{I,II} = 20°$ is in excellent agreement with our results, but we find the bands rotated approximately 5–10° clockwise. This small shift, if real, may be due to the 7 and 8 methyl groups in riboflavin, to coordination with Cu(II) in this complex, or possibly to
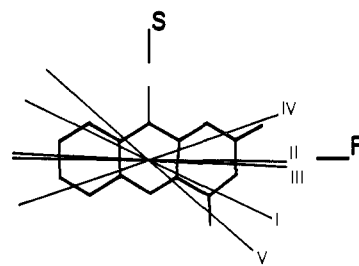


Figure 5. Most probable transition moment directions. The axis system is that of ref 22, moved to the molecular center.

differing environmental effects in the two crystals. Nonetheless, the small difference indicates that these perturbations are minor.

Circular dichroic (CD) and magnetic circular dichroic (MCD) spectra[8,26,27] have led to a greater variety of suggestions regarding the electronic structure of flavins, including the possibility of multiple transitions within most of the four resolved solution bands. Clearly our results imply that second transitions in the regions designated here as I and II be small, or very similar in polarization direction to the major bands. The presence of two strong transitions within the 280-nm solution band was not detected in the CD experiments.

Several CD experiments,[26,27] as well as calculations (vide infra), suggest the presence of a weak band between transitions II and III. Our work gives no direct evidence for such a band. If it exists, the crystal spectra indicate $f$ must not exceed ca. 0.02.

**Calculations.** Table III compares our results with four of a number of published calculations. Fox et al.,[10] using Pariser-Parr-Pople SCF techniques with limited configuration interaction, have obtained reasonably good agreement in both energy and intensity, if one makes the assignments shown in the table. These authors did not report calculated moment directions. Song, in similar calculations,[12] also reports the moment directions for transitions which we assign as I and II. Although transition energies and relative intensities are reasonably well reproduced by these calculations (which omit the 10-methyl group), they predict too large a relative angle, 42°, between transitions and in addition, predict moments which are rotated approximately 20 to 40° counterclockwise from the positions found in this crystal. Song finds in a series of calculations[6,11-13] that the predicted moment directions are quite sensitive to substituent pattern, parametrization, and extent of configuration interaction. They are also moderately sensitive to assumed geometry. Thus these measured moment directions

should provide useful additional tests of various calculational techniques.

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(20) The minor variation in relative height of the 19.7 and 21.0 kK peaks of band I and the more pronounced change in shape of band II in the (a + c/2) spectrum indicate some change in moment direction with vibrational level. The shape of band II in the (a + c/2) spectrum is a particularly sensitive function of reflectivity, however, and resolution of vibrational bands is not considered feasible.
(21) To facilitate comparison of moments measured in different crystals, or calculated and measured moments, a least-squares procedure has been developed[22] which can determine the best geometry for a given molecule by averaging the geometries obtained in several crystals, and then find the best fit of the resulting "ideal" molecule to any single crystal structure. The two most accurate flavin structures[15,23] measured to date were used to find an ideal molecule which is described in ref 22. This was in turn fitted to the position of the flavin molecule in this crystal. The ligand plane and the angles $\chi$ and $\theta$ given in Table II refer to the coordinate system determined by this ideal molecule, in which **R** runs from N(10) toward N(1), and **S** lies in the molecular plane, approximately in the direction from N(5) toward N(10). The angle between **R** and the projection of **M** onto the **R,S** plane is defined as $\theta$, and the angle between **M** and the **R,S** plane is $\chi$. The transformation between fractional crystallographic coordinates $(x,y,z)$ and reference orthonormal coordinates $(R,S,T)$ is given by

$$\begin{vmatrix} R \\ S \\ T \end{vmatrix} = \begin{pmatrix} 6.5356 & -7.6428 & -2.9742 \\ 4.5630 & 8.9089 & 3.0112 \\ -2.7717 & -3.3549 & 14.7716 \end{pmatrix} \begin{vmatrix} x \\ y \\ z \end{vmatrix}$$

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# Prediction of Core Electron Binding Energies with a Four-Parameter Equation

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**Abstract:** Chemical shifts in core electron binding energies can be predicted by the equation $\Delta E_B = aF + bR$, where the parameters $a$ and $b$ are characteristic of the class of molecule and atom to which the binding energies pertain, and the parameters $F$ and $R$ are characteristic of substituent groups. The $F$ and $R$ parameters are analogous to the Swain and Lupton $\mathscr{F}$ and $\mathscr{R}$ parameters; i.e., they measure the $\sigma$ and $\pi$ electronegativities, respectively, of substituents. However, the $F$ and $R$ values are appropriate only for processes in which a localized positive charge develops on an atom, whereas the $\mathscr{F}$ and $\mathscr{R}$ values are appropriate for ordinary chemical reactions (including both electrophilic and nucleophilic substitutions). Thus lone-pair ionization potentials and proton affinities can be correlated with $F$ and $R$ values more satisfactorily than with $\mathscr{F}$ and $\mathscr{R}$ values.

Chemical shifts in core electron binding energies can be equated to the energies of chemical reactions involving ground-state species.[1] For example, the difference between the carbon 1s binding energies of gaseous methane and gaseous carbon dioxide is practically the same as the energy of the following reaction.[2]

$$CH_4 + NO_2^+ \rightarrow NH_4^+ + CO_2$$

It has been found that, for oxygen-containing compounds (alcohols, ketones, esters, acids, etc.) and amines, shifts in the oxygen 1s and nitrogen 1s binding energies are essentially equal to the negative values of the corresponding shifts in proton affinities.[3-5] It has also been observed[6] that there is a linear correlation between the $-pK_a$ values for acids $RCH_2COOH$

and the iodine $3d_{5/2}$ binding energies of the corresponding iodides RI.

The fact that there is a close correspondence between binding energy shifts and the energies of chemical processes suggests that it should be possible to predict binding energy shifts using the same sorts of correlations and empirical parameters that are used to predict the energies of chemical processes. Indeed, it has recently been shown[7] that the carbon 1s shifts of some substituted benzenes are linearly correlated with the Hammett $\sigma$ parameters of the substituents. However, there are limitations in the use of Hammett parameters, even in the correlation of ordinary chemical data. A given set of $\sigma$ values can be used to correlate data only for similar chemical systems. To obtain a set of substituent parameters applicable