carbon, and +0.07 for each hydrogen atom. This last value is about the same as the calculated net charge for hydrogen in ethane, suggesting that the contributions of hyperconjugated valence structures, such as V, should not be substantial. The net orbital populations[26] indicate that electron density in the π system is concentrated in the O 2p atomic orbitals (1.48 e in each) at the expense of the C 2p orbitals (0.60 e each). This asymmetry in the electron distribution of the π bonds accounts for most of the net positive charge on C, and may tend to reduce the bond order somewhat from that of a "pure" triple bond. Mulliken overlap populations[26] are a thoretical measure of bond strength; the calculated C–O overlap population in $[CH_3CO]^+$, 1.15, is slightly greater than the value for carbon monoxide and about equal to that of $[CO]^+$. About 0.65 of this total is contributed by the π systems. Calculated overlap populations for C–O bonds are compared with bond lengths and infrared stretching frequencies in Table IV. Other calculated bond overlap populations for $[CH_3CO]^+$ are 0.72 for C–H and 1.03 for C–C.

The wave function was calculated by a nonempirical method[24] in which parameters for constructing the Hamiltonian matrix are derived from exact self-consistent field calculations on appropriate model compounds. The overlap and kinetic energy integrals between all atomic orbitals of the system are evaluated exactly, while the model compounds yield values for the diagonal Hamiltonian elements and the coefficients which are used in a modified Mulliken approximation to give the off-diagonal potential energy parts of the Hamiltonian. Exponents and diagonal matrix elements used in the present calculation were C(1s): 5.700, −11.284 au; C(2s): 1.625, −1.463; C(2p σ): 1.625, −0.756; C(2p π): 1.625, −0.114; O(1s): 7.700, −20.702; O(2s): 2.275, −2.573; O(2pσ): 2.275, −0.758; O(2p π): 2.275, −0.410; and H(1s): 1.200, −0.537.

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Relationship between the Absorption and Excitation Spectra and Relative Quantum Yields of Fluorescence of *all-trans*-Retinal

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Abstract: The relative quantum yields of fluorescence, ϕ_F, were calculated for different concentrations of *all-trans*-retinal. The ϕ_F were found to vary as a function of exciting wavelength. This effect was accounted for by photochemistry in competition with internal conversion in the vibrational levels of the first excited singlet state.

In a previous communication[2] we reported the fluorescence excitation spectrum of the biologically important *all-trans*-retinal. The uncorrected excitation spectrum maximized near the onset of the first absorption band of *all-trans*-retinal. The shape of the excitation spectrum was accounted for by a vibronic effect on photochemistry similar to other results obtained in this laboratory. This effect results from the fact that photochemistry is competitive with internal conversion in upper vibronic levels of the excited singlet states.[3]

Recently, other workers[4] revealed that in their investigation of *all-trans*-retinal in methylcyclohexane glasses at 77°K, the shape of the excitation spectrum varied with the concentration of *all-trans*-retinal, although at low absorbances (<0.3), they reproduced our absorption spectrum. At absorbances greater than 0.3, the maximum and short wavelength edge collapsed to yield, at absorbances greater than 2.0, an excitation spectrum similar to ours. They also concluded that the collapse of the short wavelength edge was a self-absorption effect which increased as the product of the cell path length and the concentration became large.

Experimental Section

We have repeated the fluorescence excitation spectrum of *all-trans*-retinal performing all experiments in 3-methylpentane glasses at 77°K and employing front-face emission geometry. The same kilowatt DC Zenon arc exciting source and suprasil dewar were used for all spectra. Three experiments were completed using three different fresh solutions of *all-trans*-retinal. The first experiment utilized a 2-mm suprasil cell and a solution whose absorbance was approximately 0.3; the second, a 1.0-cm suprasil cell and a solution whose absorbance was approximately 1.5; the third, a 2-mm suprasil cell and a solution whose absorbance equaled approximately 1.8. The uncorrected and corrected fluorescence excitation spectra from the first and third experiment are reproduced in Figures 1 and 2, respectively. The fluorescence excitation spectrum for the second experiment was similar to that of others.[4] Note that the excitation spectra vary depending upon concentration as would be expected. However, even at very low concentrations we could not duplicate our absorption spectrum without corrections for variation in quantum yields as a function of wavelength (see Discussion).

While we recognize that detector slit width can be a critical factor, we are confident that with small exciting slit widths (0.2 mm), a concentrated solution, a short path length cell (2 mm), large de-

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[1] Taken from a dissertation submitted in partial fulfillment of the Ph.D. degree.

[2] D. E. Balke and R. S. Becker, *J. Amer. Chem. Soc.*, **89**, 5061 (1967).

[3] R. S. Becker, E. Dolan, and D. E. Balke, *J. Chem. Phys.*, in press.

[4] G. L. Pool and A. U. Guzzo, private communication (1968). On the basis of more recent studies by these same authors, they have communicated that they are in agreement with the results as presently reported. We wish to acknowledge their stimulus for further investigation.

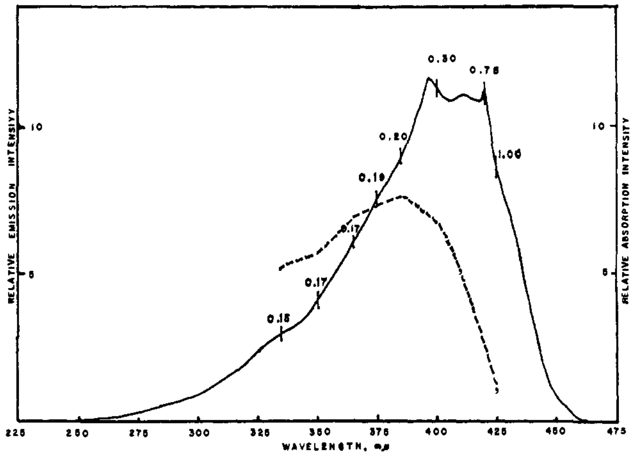

Figure 1. Fluorescence excitation spectrum (——) and corrected excitation spectrum (- - -) of *all-trans*-retinal at 77°K, absorbance approximately 0.3. The numbers represent the relative quantum yields of fluorescence at the marked wavelengths (see text).

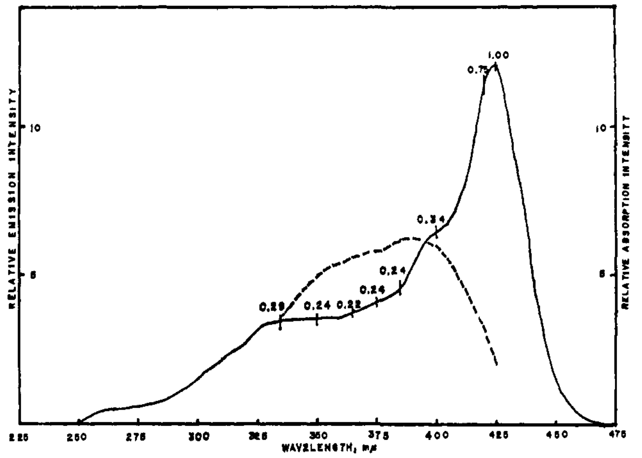

Figure 2. Fluorescence excitation spectrum (——) and corrected excitation spectrum of *all-trans*-retinal at 77°K, absorbance approximately 1.8. The numbers represent the relative quantum yields of fluorescence at the marked wavelengths (see text).

tector slit widths (2.0 mm), and a quartz lens focused front-face system, the entire emitting region is monitored. Moreover, the Franck–Condon forbidden shapes of the absorption and emission spectra permit only a small amount of band overlap where reabsorption can occur. To further avoid this problem, we monitored the emission at wavelengths equal to or greater than 500 mμ.

Results and Discussion

In order to lend further credibility to the excitation spectra, the relative quantum yields of fluorescence, ϕ_F, have been calculated throughout the first absorption band. The ϕ_F were calculated[5] as a function of light absorbed, $A(\lambda)$, the intensity of the fluorescence emission, $F(\lambda)$, and the intensity of the exciting lamp–monochromator system, $I(\lambda)$. The equation is

$$\phi_F(\lambda) = \frac{F(\lambda)}{I(\lambda)A(\lambda)}$$

$F(\lambda)$ was determined directly from the fluorescence excitation spectrum. $A(\lambda)$ was the per cent absorbance (%A) as determined from the per cent transmittance (%T) curve taking into account in the regions of %A less than 95%, that the 3-methylpentane glass–cell–dewar system adds some apparent absorption to the spectrum. $I(\lambda)$ was derived from a fluorescence excitation spectrum of rhodamine B in ethylene glycol.[6] The values of ϕ_F were normalized to a particular wavelength. This commonly was near the onset of absorption. By using this method for obtaining ϕ_F values (under the same experimental conditions) for molecules that do not undergo photochemistry (*e.g.*, 1,2-dihydronaphthalene and naphthalene) we find generally that $\phi_F = 1.00 \pm 0.05$ (standard deviation). On the basis of these results, we expect a similar error for the data reported herein. Furthermore, in order to use these ϕ_F values to reproduce absorption spectra, one must solve the previous equation for %T or absorbance.

From Figures 1 and 2, it is evident that ϕ_F decreases as the wavelength decreases, then appears to level off. This effect has been noted in other cases and studied in considerably greater detail.[3] We interpret the decrease in ϕ_F as arising from a competition between photochemistry and internal conversion between the vibrational levels of the first excited singlet state.[3] Using the ϕ_F values, we can reproduce the first absorption band with a maximum at approximately 383 mμ (Figures 1 and 2). This is in conformity with the known low temperature absorption data.[2] Generally, there is good agreement between the two sets of data. The difference in the data at 335 mμ is clearly outside the expected standard deviation which, of course, is always possible. However, the difference is sufficiently great that some additional factor(s) to that noted above may be contributing. For example, because of the high concentration of retinal, more photoproduct[2] may be produced in this region of absorption and thereby alter the intensity of fluorescence in the region monitored.

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