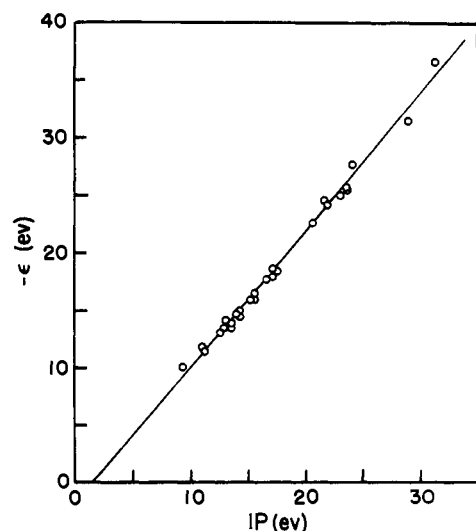
Figure 2. MO Energies for $\text{CH}_3\text{-X}$ Compounds.Figure 3. Correlation between ϵ and IP.

is developed. With both $\text{X} = \text{NH}_3^+$ and O^- , all energy levels are markedly perturbed.

The photoelectron spectra of many of these compounds have been examined and the ionization potentials determined.⁸ It was previously found that the calculated molecular orbital energies for a series of alkenes were linearly related to the vertical ionization potentials, provided that a nonzero intercept was allowed.⁹ The same is true in this case, and the correlation holds for both s- and p-type orbitals (Figure 3). The relation between the quantities is given by

$$-\text{IP} = 0.83\epsilon - 1.5$$

This is essentially the same as that found for the alkenes.

The separation of the charge shifts into the σ and π components and the extension of these calculations to ethyl derivatives will be presented at a later time.

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References and Notes

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Photoselected Fluorescence Detected Circular Dichroism

Sir:

We report the first quantitative measurement of photoselected fluorescence detected circular dichroism (FDCCD). In this measurement the optical activity of a chromophore is obtained by measuring the difference in fluorescence intensity for left and right circularly polarized excitation under conditions where rotation before emission is negligible. It was first pointed out by Ehrenberg and Steinberg that for these conditions the FDCCD spectrum is not predicted by the simple theory of Turner et al.¹⁻³ Tinoco et al. subsequently developed a theory for this photoselected case.⁴ According to this theory, the FDCCD spectrum of the unoriented molecule can be obtained if a particular polarization of the fluorescence is detected. We have measured the FDCCD spectrum of 10-*d*-camphorsulfonic acid (CSA) and of morphine in glycerol and find reasonable agreement with this theory.

When the detector is perpendicular to the exciting light, and a linear polarizer is placed in front of it, the following expression can be derived for the measured FDCCD signal when fluorophore rotation is negligible:^{1,2,4}

$$\theta_F^{\pm} = -14.32 \times \left[\frac{8R(1 + \cos^2 \phi) + (8R_{33}/3)(2 - 3 \cos^2 \phi)}{D(4 - \cos^2 \phi) - (D_{33}/3)(2 - 3 \cos^2 \phi)} - 2R_1 \right] \quad (1)$$

ϕ is the angle that the detected polarization makes with the axis perpendicular to the excitation beam (i.e., when the polarizer is perpendicular to the excitation beam, $\phi = 0^\circ$), R and D are the average rotational and dipole strengths, respectively, and R_{33} and D_{33} are the rotational and dipole strengths along the direction of the emission transition moment. The quantity R_1 is given by

$$R_1 = \frac{\Delta A}{2A} - \frac{2.303 \Delta A \times 10^{-4}}{2(1 - 10^{-4})} \quad (2)$$

where A is the absorbance of the sample and ΔA is the absorbance for left circularly polarized light minus that for right circularly polarized light. Equation 1 predicts that the FDCCD spectrum can be a function of polarizer angle and that $\theta_F^{\pm} = -14.32(4R/D - 2R_1)$ when $\phi = 35.25^\circ$. The values of $4R/D$ and R_1 can be obtained by measuring the circular dichroism (CD) and absorption spectra of the sample if only one species is present. Thus, comparison of the FDCCD spectrum predicted

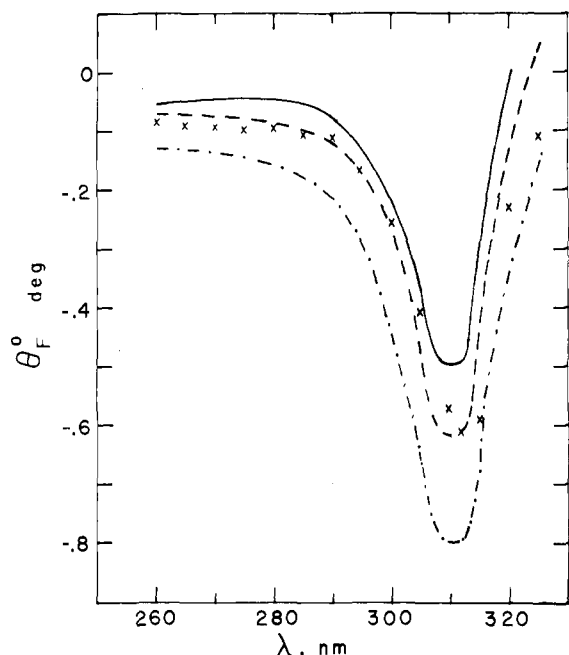


Figure 1. Photoslected FDCD spectra of 0.43 M 10-d-camphorsulfonic acid in glycerol: (—) $\phi = 0^\circ$; (---) $\phi = 35^\circ$; (-·-) $\phi = 90^\circ$; (x) spectrum predicted from CD and absorption spectra. Base line solution was 1.0×10^{-4} M DL-tryptophan in water. Filter was Corning 4-72 and cell path length was 1 mm.

from eq 1 with the experimental spectrum provides a convenient test for the theory.

The apparatus is a modified version of the previously described instrument.^{1,5} A linear polarizer on a rotatable mount is placed between the cell and the photomultiplier. Initial experiments on optically inactive, fluorescent dyes dissolved in glycerol gave large artifactual ellipticities in the measurement for $\phi = 0^\circ$. These appear to be due to imperfect circularly polarized light. The circular polarization has been improved by mounting the Pockel's cell modulator on a kinematic mount for precise alignment and replacing the original switching voltage on the cell with an accurate high voltage square wave. This has reduced the artifact from ~ 200 mdeg to ~ 50 mdeg for $\phi = 0^\circ$. The artifact is negligible for $\phi = 90^\circ$. The instrument is calibrated with CSA in water as described previously.⁵ The glycerol is certified Spectroanalyzed grade from Fisher and is redistilled to remove interfering fluorescence impurities.

The fluorescence of CSA in glycerol is highly polarized as indicated by a linear depolarization ratio, $(F_{\parallel} - F_{\perp}) / (F_{\parallel} + F_{\perp})$, of 0.5 at the long wavelength edge of the excitation spectrum.⁶ Thus photoselection effects will be present. The FDCD spectra of CSA in glycerol for $\phi = 0, 35,$ and 90° are shown in Figure 1. The maximum ellipticity is on the order of 800 mdeg, so that artifactual contributions are negligible. The spectra shown in Figure 1 are dependent on ϕ . This is not true for CSA dissolved in water where the depolarization ratio is only 0.02. According to eq 1, the CD and absorption spectra of CSA can be used to predict the FDCD spectrum for $\phi = 35.25^\circ$. Points calculated from eq 1 are shown in Figure 1. These points agree with the spectrum measured for 35° within experimental error. Thus there is good agreement between experiment and theory.

One of the major potential applications of FDCD is to study local conformations in macromolecules. In some cases, this will involve the photoselected case. The above results demonstrate that it is possible to obtain quantitative information by choosing the polarization of the detected fluorescence. However, measurements on phenylalanine tRNA and human serum albumin indicate that typical signals will be on the order of 50

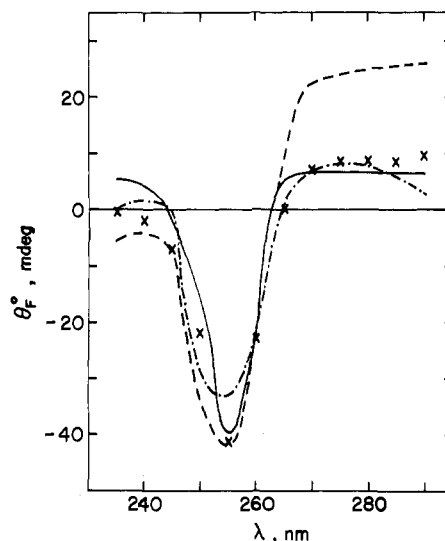


Figure 2. Photoslected FDCD spectra of 4.05×10^{-3} M morphine in 90% glycerol-10% water (v/v), 0.1 M H_2SO_4 : (—) $\phi = 0^\circ$; (---) $\phi = 35^\circ$; (-·-) $\phi = 90^\circ$; (x) spectrum predicted from CD and absorption spectra. Filter was Wratten 18-A and cell path length was 1 mm.

mdeg.^{7,8} Thus artifacts can be a serious problem. Measurements on optically inactive, fluorescent dyes dissolved in glycerol indicate that the artifacts are similar (± 15 mdeg) for concentrated (absorbance > 5) and dilute solutions. This is reasonable, if the artifact arises from imperfect circularly polarized light that changes between the left and right cycles. In contrast, eq 1 predicts that the FDCD signal at high concentrations approaches zero for $\phi = 35.25^\circ$. It also indicates that, for any ϕ , the difference between the FDCD measured for a concentrated and dilute sample containing one species is given by twice the second term of eq 2. The different response of artifact and signal to concentration can be used to further reduce and correct for the artifact. This method has been tested by measuring the FDCD of morphine in glycerol.

The depolarization ratio of morphine in 90% glycerol is 0.3 in the long-wavelength band, so that photoselection effects are present. A concentrated solution of 8×10^{-2} M was used as the base line. This corresponds to an absorbance of 11.2 at the 286-nm maximum. The Pockel's cell orientation was adjusted to give a minimum difference between the base lines at $\phi = 35^\circ$ for the concentrated sample and for sodium fluorescein in water. Then the spectrum of a 4×10^{-3} M sample was recorded. The differences of spectra measured for concentrated and dilute morphine are shown in Figure 2, along with the result predicted from the CD and absorption spectra of the dilute sample. The 0 and 90° spectra are essentially coincident and agree well with the result predicted. This does not mean that the artifact has been completely eliminated, however. The 35° measurement gives a 270–290-nm band with an average ellipticity of 25 mdeg, rather than the expected 9 mdeg. Thus the absolute error in the measurement is roughly ± 15 mdeg.

The importance of this work is that it provides the first experimental verification that photoselected FDCD can be studied by choosing the polarization of the detected fluorescence. Artifacts have been reduced to a level of ~ 15 mdeg. Preliminary results on human serum albumin indicate that this is sufficient to determine $4R/D$ for its single fluorescent tryptophan to $\pm 30\%$.⁹ Other proteins should give similar results. Since many conformational arguments require only the sign of the optical activity, this should provide much useful information.

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