

Figure 1. Apparent ^{13}C transverse relaxation rates *vs.* Carr-Purcell pulse repetition rate $(2\tau)^{-1}$ for 60% enriched $^{13}\text{CH}_3\text{I}$ and $^{13}\text{CH}_3\text{COOCD}_3$. The theoretical curves assume the model of a two-spin AX system, where $R_{2\text{C}}^0 = R_{1\text{C}}$ and \bar{R} is chosen to agree with the slow pulse limit. This model is based on $\Delta I_{\text{H}} = \pm 1$ transition only; however, inclusion of $\Delta I_{\text{H}} = \pm 2$ would improve the agreement in the region above $(2\tau)^{-1} = 100 \text{ sec}^{-1}$.

of A causing irreversible decay of the magnetization in the transverse plane. In particular, for $^{13}\text{CH}_3\text{I}$ and $^{13}\text{CH}_3\text{COOCD}_3$, whose $T_{1\text{H}}$'s are dominated by intramolecular dipolar effects, the scalar contribution to $R_{2\text{C}}$ is determined by the rate, \bar{R} , averaged over all states, of such H transitions. One might guess that $\bar{R} = 3R_{1\text{H}}$; however, $R_{1\text{H}}$ is based not only on the number of such transitions, but also on the ability of these to produce thermal equilibrium. Thus, for example, a $\Delta I_{\text{H}} = \pm 2$ transition is a more effective $T_{1\text{H}}$ process than $\Delta I_{\text{H}} = \pm 1$, whereas \bar{R} considers both equally. This implies that $\bar{R} < 3R_{1\text{H}}$ for a CH_3 system; to be more quantitative is beyond the scope of this communication.

The theory for coupled AX systems presented by Gutowsky, *et al.*,⁸ predicts a sigmoidal plot^{8,9} of $R_{2\text{C}}$ *vs.* $(2\tau)^{-1}$ for Carr-Purcell measurements with π pulse repetition rates $(2\tau)^{-1}$. Two limits are predicted. For rapid pulse rates

$$R_{2\text{C}} \rightarrow R_{2\text{C}}^0 \quad (2\tau)^{-1} \gg A \quad (2)$$

where $R_{2\text{C}}^0$ is the transverse relaxation rate in the absence of scalar coupling effects and is often very close to $R_{1\text{C}}$. For slow pulse rates

$$R_{2\text{C}} \rightarrow R_{2\text{C}}^0 + \bar{R} \quad (2\tau)^{-1} \ll A \quad (3)$$

and a damped oscillation is predicted^{8,9} for $(2\tau)^{-1} \lesssim J$.

In Figure 1, $R_{2\text{C}}$ for $^{13}\text{CH}_3\text{I}$ and $^{13}\text{CH}_3\text{COOCD}_3$ is plotted *vs.* $(2\tau)^{-1}$. The values of J_{CH} are 152 and 130 Hz, respectively, and there is good qualitative agreement between these curves and those predicted by theory.^{8,9} A maximum in $R_{2\text{C}}$ is predicted for $(2\tau)^{-1} = 2J/3$; we observe a maximum in the vicinity of this value. We also find a less rapid decrease in $R_{2\text{C}}$ at higher pulse rates, which is consistent with the occurrence of intramolecular $\Delta I_z = \pm 2$ transitions between proton spin states resulting in coupling energy changes of $\pm 2J$. Pulse repetition rates faster than those experimentally available to us [$(2\tau)^{-1} > 1000 \text{ sec}^{-1}$] are necessary to pulse these effects completely away. An unsatisfactory feature of this graph is that the values of $R_{2\text{C}}$ seem to plateau above $R_{1\text{C}}$ at the higher pulse rates. For

(9) A. Allerhand and H. S. Gutowsky, *J. Chem. Phys.*, **42**, 1587 (1965).

$^{13}\text{CH}_3\text{I}$ the scalar coupling to ^{127}I may result in $R_{2\text{C}}^0 > R_{1\text{C}}$; however, the higher limiting value of $R_{2\text{C}}^0$ observed for $^{13}\text{CH}_3\text{COOCD}_3$ cannot be explained this way. At these high pulse rates we may be exceeding the duty cycle of our transmitter; we plan to investigate compounds where the fast pulsing limit should occur well within our instrumental capabilities. In the slow pulsing limit our experimental values indicate, as expected, that $\bar{R} < 3R_{1\text{H}}$, when $R_{2\text{C}}^0 = R_{1\text{C}}$. Corrections required to account for scalar coupling effects from I in $^{13}\text{CH}_3\text{I}$ would decrease \bar{R} even further. The small ^{13}C -D coupling in $^{13}\text{CH}_3\text{COOCD}_3$ can in principle contribute to $R_{2\text{C}}$, but any effect has been pulsed away in these measurements [$(2\tau)^{-1} \geq 10 \text{ sec}^{-1}$].

As expected, $T_{2\text{C}}$ of $^{13}\text{CS}_2$ was found to be independent of the Carr-Purcell time. Experiments in this laboratory¹⁰ have shown that spin rotation is the dominant relaxation mechanism for $^{13}\text{CS}_2$ at 15 MHz, in agreement with data reported elsewhere.¹¹

In summary, it appears that the ultimate resolution in ^{13}C spectra will be of the order of the proton line widths, as long as eq 1 is satisfied for all coupled nuclei. Furthermore, the advantage predicted by the multiple-pulse, refocusing method^{1,2} over the normal FT technique will be greatly reduced, since the promised sensitivity enhancement² was based on the assumption that $T_2 \approx T_1$.

The foregoing remarks relate to undecoupled spectra only. For pseudorandom proton noise decoupling the ^{13}C line widths should be a function of the H_2 rf level. At lower rf power, for which $A \gtrsim \tau_{\text{H}}^{-1}$, where τ_{H} is the effective lifetime of the proton spin states in the presence of H_2 , $R_{2\text{C}}$ should increase and reach a maximum as the rf power increases. For rf power sufficiently high that $A \ll \tau_{\text{H}}^{-1}$, $R_{2\text{C}}$ should then decrease toward $R_{2\text{C}}^0$. The refocusing techniques might therefore still be useful under conditions of strong, random proton decoupling.

(10) T. C. Farrar, S. J. Druck, R. R. Shoup, and E. D. Becker, to be published.

(11) In general, our I_2 measurements are reproducible to $\sim \pm 5\%$; however, systematic errors may increase for very fast pulse rates and very long T_2 's. The latter may account for the difference between $T_{1\text{C}}$ and $T_{2\text{C}}$ for $^{13}\text{CS}_2$.

R. R. Shoup*

National Institutes of Health
Bethesda, Maryland 20014

D. L. VanderHart

National Bureau of Standards
Washington D. C. 20234

Received November 30, 1970

Polarized Single-Crystal Absorption Spectrum of Cytosine Monohydrate

Sir:

In this communication we present polarized absorption spectra of single crystals of cytosine monohydrate. Callis and Simpson have recently reported a polarized reflectance study on cytosine monohydrate and 1-methylcytosine.¹ The results of these authors are in excellent agreement with our own, but our more sensitive direct absorption measurements using a microspectrophotometer permit additional and more quantitative

(1) P. R. Callis and W. T. Simpson, *J. Amer. Chem. Soc.*, **92**, 3593 (1970).

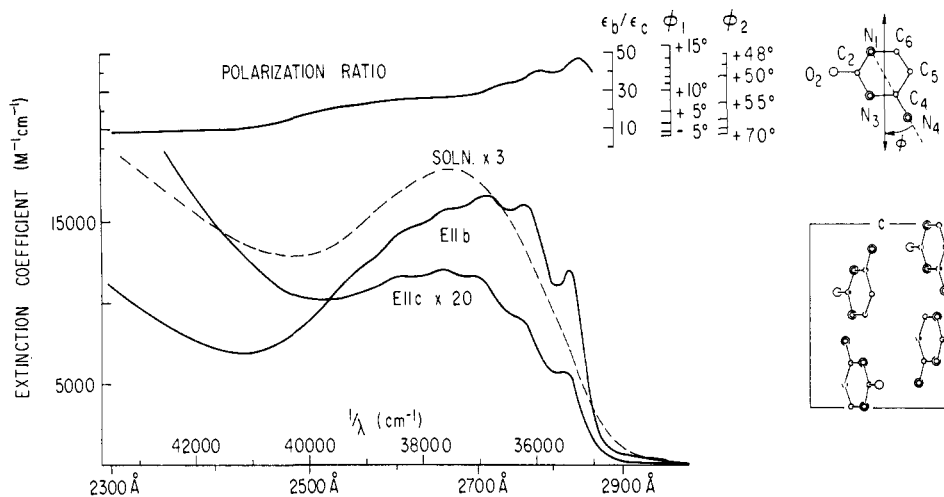


Figure 1. Polarized absorption spectrum of cytosine monohydrate single crystal measured with a microspectrophotometer at room temperature with light incident normal to the (100) crystal face. The c -axis polarized spectrum has been multiplied by a factor of 20 and the aqueous solution spectrum by 3. The polarization ratio is the ratio of the b - to c -axis polarized decadic molar extinction coefficients, ϵ_b/ϵ_c . If all the intensity is polarized parallel to the molecular planes, ϕ_1 and ϕ_2 describe the two possible oriented gas electric dipole transition moment directions consistent with each polarization ratio (see text). In calculating molecular transition moment directions from the observed polarization ratios, no correction was made to take into account the fact that the c -crystal axis does not correspond to a principal optical direction¹⁰ (see J. W. Rohleder and T. Luty, *Mol. Crystals*, **5**, 145 (1968)).

conclusions. With these studies experimentally determined transition moment directions are available for derivatives of four of the five common nucleic acid bases—uracil,² thymine,³ cytosine, adenine.³⁻⁶ This polarization information is not only required for theoretical investigations, but it also forms the basis for investigating the structure of nucleic acids in solution by linear dichroism methods.⁷

Figure 1 shows the spectrum of the (100) face of the cytosine monohydrate crystal with the incident light polarized parallel to the b and c crystal axes.⁸ Also presented are the variation with wavelength of the polarization ratio, ϵ_b/ϵ_c , and an aqueous solution spectrum. The projection of the four cytosine rings of the monoclinic unit cell (space group $P2_1/c$) onto the (100) face is indicated, and the nearly planar rings are all parallel.⁹ The c axis makes an angle of 62° with the best ring plane, while the b axis makes an angle of less than 1° with this plane. Consequently, transitions polarized perpendicular to the molecular planes will appear only in the c -axis spectrum, whereas transitions polarized parallel to the molecular planes will in general appear in both the b - and c -axis spectra. It is evident that most of the intensity in the region

of the longest wavelength $\pi \rightarrow \pi^*$ transition is b -axis polarized and is, therefore, polarized parallel to the molecular planes. The b - and c -polarized spectra appear so similar that the bulk of the intensity observed along the c axis also arises from in-plane-polarized transitions (*vide infra*).

The isotropic oscillator strength for the longest wavelength transition, calculated from the crystal intensities,¹² is approximately 0.13. This is also the value estimated from the aqueous solution oscillator strength. This comparison indicates that any crystal-induced intensity changes are small.

The observed vibrational interval is $750 \pm 100 \text{ cm}^{-1}$ which could correspond to a single progression in the totally symmetric ring mode observed in the ground electronic state of cytosine monohydrate crystals at 796 cm^{-1} .¹³ From the close agreement of the vibrational band maxima in the two polarizations it is clear that the coupling between molecules is weak.¹⁴ The 0,0 band at about $35,500 \text{ cm}^{-1}$ is sharp enough to estimate an apparent Davydov (exciton) splitting of $50\text{--}75 \text{ cm}^{-1}$, with the b -polarized crystal state at lower energy. A calculation of the Davydov splitting in the dipole approximation,¹⁴ using the ring centers for the position of the point transition dipole and including interactions with all molecules within 100 \AA , predicts a 100-cm^{-1} splitting of the 0,0 band, with the b -polarized state again at lower energy.¹⁵

In order to obtain *free* molecule electric dipole transition moment directions we shall analyze the polarization data in terms of a collection of noninteracting molecules, *i.e.*, the crystal is considered to behave

(12) The isotropic oscillator strength for the crystal was calculated from the relation $f_{iso} = 1/3(f_a + f_b + f_c) = 1/3(0.04 + 0.34 + 0.011) = 0.13$. The a -polarized oscillator strength, $f_a = 0.04$, was calculated using a f_b/f_a polarization ratio of 8:1 from the Callis and Simpson reflectance data on the (001) crystal face. The above relation for f_{iso} is only approximately correct since the a , b and c crystal axes are not mutually orthogonal ($\beta = 99^\circ 42'$).

(13) R. C. Lord and G. J. Thomas, *Spectrochim. Acta, Part A*, **23**, 2551 (1967).

(14) D. P. Craig and S. H. Walmsley, "Excitons in Molecular Crystals," W. A. Benjamin, New York, N. Y., 1968.

(15) We would like to thank Professor Howard DeVoe for providing us with the computer program to evaluate dipole-dipole lattice sums.

(2) W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, **53**, 2164 (1970).

(3) R. F. Stewart and N. Davidson, *ibid.*, **39**, 255 (1963).

(4) R. F. Stewart and L. H. Jensen, *ibid.*, **40**, 2071 (1964).

(5) H. H. Chen and L. B. Clark, *ibid.*, **51**, 1862 (1969).

(6) H. H. Chen and L. B. Clark, private communication.

(7) E. Charney in "Procedures in Nucleic Acid Research," Vol. 2, G. L. Cantoni and D. R. Davies, Ed., Harper and Row, New York, N. Y., in press.

(8) Single crystals of extremely high optical quality as thin as 0.1μ with measuring areas up to $100 \times 100 \mu$ were grown between a quartz slide and coverslip by evaporation from aqueous solution. The (100) crystal face was identified by zero-level X-ray precession photographs,⁹ conoscopic examination, and measurement of interfacial angles.¹⁰ Optical density, relative retardation, and refractive index measurements were made as previously described.² The refractive indices at 5890 \AA are $n_b = 1.785 \pm 0.003$ and $n_c = 1.486 \pm 0.004$. A small reflection correction ($< 5\%$) to the b -axis optical densities was made, based on the reflectance data of Callis and Simpson.^{1,11} The total experimental uncertainty in the b - and c -axis extinction coefficients, determined from optical density and relative retardation measurements on 14 crystals, is 10 and 4%, respectively.

(9) G. A. Jeffrey and Y. Kinoshita, *Acta Crystallogr.*, **16**, 20 (1963).

(10) H. A. Rose, *Anal. Chem.*, **27**, 158 (1955).

(11) A. Bree and L. E. Lyons, *J. Chem. Soc.*, 2662 (1956).

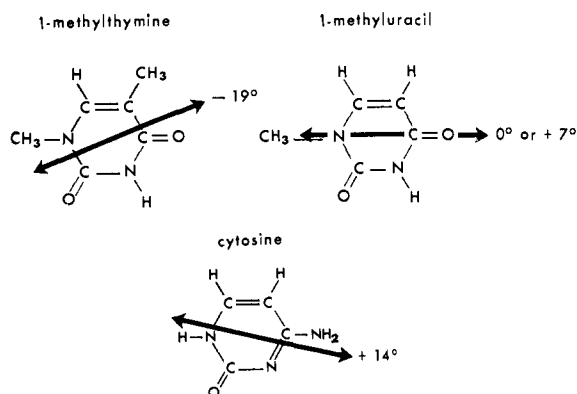


Figure 2. Transition moment directions for the longest wavelength $\pi \rightarrow \pi^*$ transition in 1-methylthymine (all vibronic components),³ 1-methyluracil (0,0 band),² and cytosine monohydrate (0,0 band) as derived from polarized single-crystal absorption measurements. The angles are measured from the N_1-C_4 direction.

like a so-called oriented gas. The absolute intensities and the small Davydov splittings support such a treatment. Assuming that all the intensity in the c -axis spectrum is polarized parallel to the molecular planes, the polarization ratio for each vibronic component is consistent with *two* possible in-plane molecular directions for the transition moment. These two possible molecular directions are expressed in Figure 1 as the angles, ϕ_1 and ϕ_2 , measured relative to the N_1-C_4 direction. Callis and Simpson¹ chose ϕ_1 as the correct alternative on the basis of their results on the 1-methylcytosine crystal. ϕ_1 , calculated from the ratio of the b - to c -polarized intensities for all the vibronic components of the first transition (37,000 to about 41,000 cm^{-1}), is $+10^\circ$, in excellent agreement with the $+9^\circ$ value estimated from their reflectance data on the (001) face of cytosine monohydrate. However, the best experimental value to be compared with the results of theoretical calculations is $\phi_1 = +14 \pm 1^\circ$ (Figure 1) for the 0,0 band, since this region of the spectrum should be least influenced by intermolecular interactions and molecular vibrations which can "mix" Born-Oppenheimer free-molecule electronic states.

The second transition, centered at about 44,000 cm^{-1} in aqueous solution,¹⁶ dominates the absorption above 42,000 cm^{-1} in the crystal, where the polarization ratio levels off at about 8.5:1. This polarization ratio corresponds to a ϕ_1 of $-5 \pm 3^\circ$, again in good agreement with the Callis and Simpson result of $-1 \pm 10^\circ$.

Figure 2 summarizes the experimentally determined transition moment directions for the first $\pi \rightarrow \pi^*$ transition in the three common nucleic acid pyrimidines. Theoretically predicted directions for uracil and thymine,¹⁷⁻²¹ including a recent *ab initio* calculation on thymine,²² are generally within about 10° of the observed directions for 1-methyluracil² and 1-methylthymine.³ However, π -electron calculations on cytosine,

(16) H. Morita and S. Nagakura, *Theoret. Chim. Acta*, **11**, 279 (1968).

(17) H. DeVoe and I. Tinoco, *J. Mol. Biol.*, **4**, 518 (1962).

(18) T. A. Hoffman and J. Ladik, *Advan. Chem. Phys.*, **7**, 84 (1964).

(19) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Theoret. Chim. Acta*, **5**, 53 (1966).

(20) H. Berthod, C. Giessner-Prettre, and A. Pullman, *Int. J. Quantum Chem.*, **1**, 123 (1967).

(21) J. Ladik and K. Appel, *Theoret. Chim. Acta*, **4**, 132 (1966).

(22) L. C. Snyder, R. G. Shulman, and D. B. Neumann, *J. Chem. Phys.*, **53**, 256 (1970).

which require more extensive parameterization than uracil or thymine because of the greater variety of chemical bonds, predict directions which generally differ from experimental values by about 60° ;^{16-21,23} the maximum possible difference between theory and experiment is 90° .

The good agreement between the transition moment directions calculated from the reflectance data for the (001) crystal face, which is nearly parallel to the molecular planes, and our absorption data for the (100) crystal face support the assumption in the previous analysis that all of the c -axis intensity is polarized parallel to the molecular planes. Unlike the case of 1-methyluracil, where we obtained a spectrum polarized almost exactly perpendicular to the molecular planes,² we cannot locate $n \rightarrow \pi^*$ transitions below 44,000 cm^{-1} in cytosine monohydrate. Rich and Kasha found an $n \rightarrow \pi^*$ transition at about 36,000 cm^{-1} in polycytidylic acid from polarized absorption measurements on oriented films.²⁴ If an $n \rightarrow \pi^*$ transition in cytosine monohydrate were of comparable energy, intensity, and polarization we would have observed it as a distinct band in the c -axis polarized spectrum.

(23) C. Nagata, A. Imamura, Y. Tagashira, and M. Kodama, *Bull. Chem. Soc. Jap.*, **38**, 1638 (1965).

(24) A. Rich and M. Kasha, *J. Amer. Chem. Soc.*, **82**, 6197 (1960).

(25) Department of Chemistry, The City College of the City University of New York, New York, N. Y. 10031.

Thomas P. Lewis,*²⁵ William A. Eaton*

Laboratories of Molecular Biology and Physical Biology
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland 20014

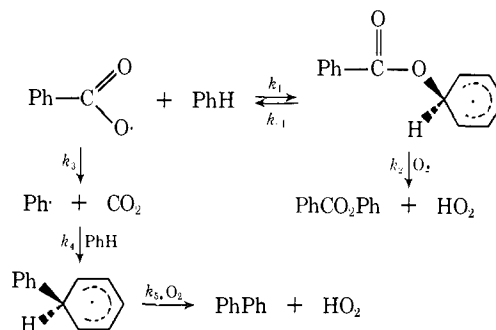
Received January 11, 1971

Reversible Addition of Benzoyloxy Radicals to Benzene

Sir:

Irradiation of benzoyl peroxide or benzil in benzene in the presence of oxygen gives phenyl benzoate^{1,2} in yields which increase with oxygen concentration. These observations have been accounted for by assuming that the addition of benzoyloxy radicals to benzene is reversible, Scheme I.^{1,2} This reversible

Scheme I. The Fate of Benzoyloxy Radicals in Benzene



addition provides the only analogy in free-radical chemistry for the suggestion that the major path of deactivation in benzene of carbonyl triplet states with n, π^* electronic character involves reversible formation of adduct biradicals.³⁻⁶ In the following, evidence is pre-

(1) T. Nakata, K. Tokumaru, and O. Simamura, *Tetrahedron Lett.*, 3303 (1967).

(2) J. Satiel and H. C. Curtis, *Mol. Photochem.*, **1**, 239, 461 (1969).