studies[7,8] suggest an energy difference of 7–11 kcal between these acetic acid cis and trans forms, and Table I shows that for $H\ddot{C}COOH$ the analogous separation is 7.7 kcal.

With the trans–trans form established as the lowest energy triplet conformer, an optimization of the three carbene geometrical parameters was carried out at the DZ SCF level of theory, and the results are seen in Figure 1. The resulting total energy is −226.47253 hartrees, or 0.00133 hartree below the energy with the assumed triplet geometry. For the singlet state, a preliminary conformational map was obtained, assuming $r(\mathrm{CC}) = 1.50$ Å, $r(\mathrm{CH}) = 1.10$ Å, and $r(\mathrm{H\ddot{C}C}) = 105°$, typical singlet carbene structural parameters.[9] These singlet conformational results are seen in Table I and show the expected[4,6–8] result (also found for the triplet state) that the hydroxyl H prefers to lie trans to the carbene carbon atom. What was not expected by us is the nonplanar equilibrium geometry predicted for the lowest singlet state. The potential well (for $\phi = 0°$) is rather symmetric with respect to the dihedral angle θ, so it is clear that the optimum angle is close to $\theta = 90°$. On the basis of this and the corresponding triplet model geometries, the singlet–triplet separation is predicted to be 30.0 kcal. Partial optimization of the singlet structure was carried out, is seen in Figure 1, and gives a total energy of −226.42480 hartrees. Comparison with the analogous triplet energy reported above provides a singlet–triplet separation of 29.9 kcal.

For the parent CH_2, the above DZ SCF approximation yields a singlet–triplet separation (E) that is $\sim$22 kcal too large.[10] In this regard, a two-configuration SCF treatment of the singlet state is more appropriate, and this yields a DZ ΔE of 21.1 kcal for $H\ddot{C}COOH$. Addition of carbene carbon d functions reduces the singlet–triplet separation further to 11.9 kcal. Configuration interactions involving single and double excitations (19 530 configurations for the ^1A state, 6635 for the $^3A''$ state) yielded total energies of −226.63428 and −226.65360 hartrees, and hence $\Delta E = 12.1$ kcal. Finally, use of the Davidson unlinked cluster correction[11] reduced ΔE to 7.6 kcal, remarkably close to the value obtained by empirically subtracting 22 kcal from the simplest theoretical prediction of 29.9 kcal.

Moreover, direct theoretical studies of CH_2 at a level of theory essentially comparable to the best results reported here provide a reasonable singlet–triplet separation for methylene. Specifically, Bauschlicher and Shavitt[12] predict a separation of 12.4 kcal in this way while the most exhaustive theoretical studies[10,12,13] suggest $\Delta E \sim 11$ kcal. Thus, there is independent evidence of the validity of the present theoretical treatment of singlet–triplet separations, which supports the final prediction of a gap of $\sim$7 kcal for $H\ddot{C}COOH$. This small separation, combined with the relative nonreactivity[14] of triplet carbenes, could explain why carbalkoxycarbenes appear[2] to be singletlike on the basis of their reactions. However, a simpler explanation is that the normal course of reaction for these carbenes appears to involve generation and fast reaction of the singlet states.[15]

Carbohydroxycarbene may also be viewed in light of a number of recent studies[9,16–18] on the effects of heteroatom substitution on the singlet–triplet splitting in methylene. Bauschlicher et al.[9] and Harrison et al.[17] emphasize the stabilization of the singlet relative to the triplet state due to σ-inductive withdrawal of electrons by electronegative substituents. The inductive withdrawal of the σ electrons primarily localized on the carbenic carbon stabilizes the singlet in which this orbital is doubly occupied relative to the triplet where it is singly occupied. Baird and Taylor[16] and Feller et al.[18] have stressed the importance of considering the donation of out-of-plane lone pairs on heteroatoms such as oxygen into the empty (in the singlet) π orbital on carbon. Carbohydroxycarbene is somewhat different from simpler carbenes such as $C(OH)_2$[18] in that the optimum geometry has the HCC and CCO planes nearly orthogonal to avoid repulsion between the carbene carbon and the oxygen lone pair orbitals. The doubly occupied orbital on the carbene center is now oriented to donate electron density to the slightly positive carbonyl carbon. The empty molecular orbital on the carbenic center is now a σ-type orbital which may, in fact, accept electron density from the lone pair on oxygen. The net effect of these two interactions should be a slight stabilization of the singlet state relative to the triplet state in carbohydroxycarbene as compared to methylene.

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Assignment of the Anomalous Resonance Raman Vibrations of Bathorhodopsin

Sir:

The resonance Raman (RR) spectrum of bathorhodopsin, the primary photochemical intermediate in vision, is dominated by three intense lines near 854, 875, and 922 cm^{-1} which are unique to this intermediate (Figure 1A).[1] These lines are characteristic of the retinal chromophore conformation and must be explained by any model for the structure of bathorhodopsin.[2,3] Our previous work with methyl-deuterated and demethylbathorhodopsin analogues suggested that they are due to hydrogen out-of-plane (HOOP) wagging vibrations whose intensities are induced by small relative chain twists between ground and resonant excited states.[4] In this communication, we definitively assign the 875- and 922-cm^{-1} bathorhodopsin lines to specific HOOP modes. This assignment is based on Raman spectral shifts in analogue bathorhodopsins whose retinal chromophores are deuterated at specific positions on the polyene chain.

The synthesis of 11,12-dideuterioretinal (**6**) and 10-monodeuterioretinal (**11**) is summarized in Scheme I. The acetylenic C_{16} carbinol[5] **2** was prepared in 98% yield from β-ionone (**1**) via a Grignard. Treatment of **2** with acetic anhydride and 4-dimethylaminopyridine gave the corresponding acetate (**3**), which

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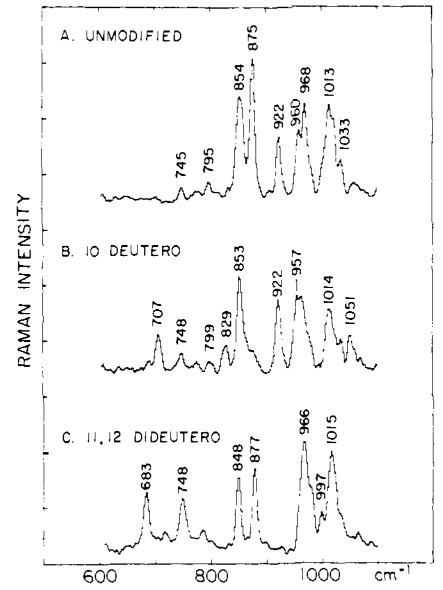
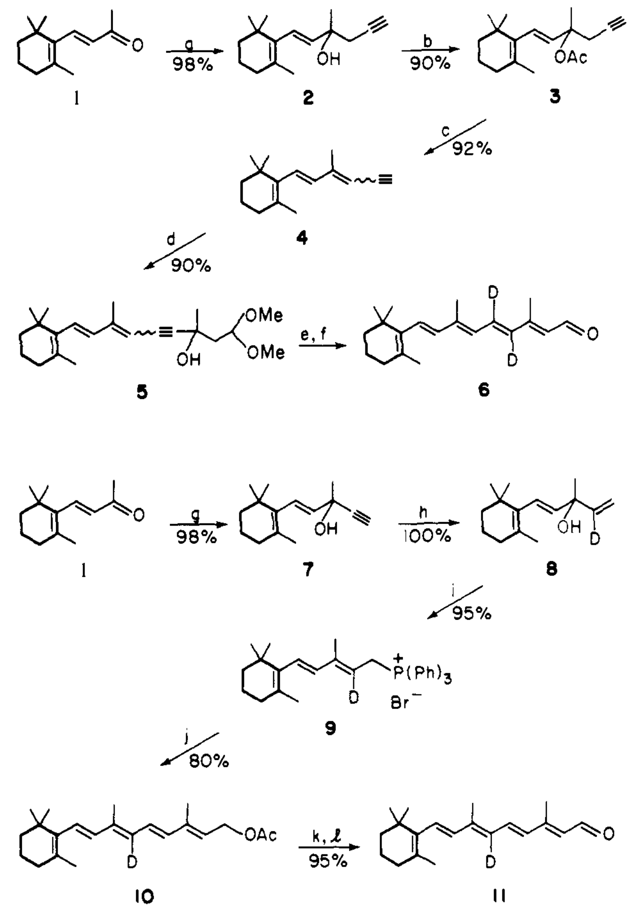


Figure 1. Resonance Raman spectra of steady-state mixtures of unmodified (A), 10-monodeuterio (B), and 11,12-dideuterio (C) pigments in Ammonyx LO at 77 K probed with 488-nm light. Spectral slit widths are 5–6 cm^{-1}.

was deacetylated to the C_{16} hydrocarbon (**4**) with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) activated by $NaNH_2$. The acetal[6] **5** was obtained by reaction of **4** with $CH_3COCH_2CH(OCH_3)_2$ in the presence of BuLi at −60 °C. Reduction of **5** with $LiAlD_4$ and workup with D_2O and acid treatment gave a mixture of isomers (**6**).[7] After purification by column chromatography (silica gel 60, 4% ether in 40–60 °C petroleum ether) the mixture was separated by high-pressure liquid chromatography (high-pressure LC) (Partisil 5, 20% ether in pentane) into the all-trans, 9-cis, and 13-cis isomers. The 11-cis isomer was obtained by irradiation of the all-trans isomer in CH_3CN and separation by high-pressure LC. In the 10-monodeuterio synthesis, ethynyl-β-ionol[8] (**7**) was prepared in 98% yield from β-ionone (**1**) via a Grignard. Reduction of **7** with $LiAlD_4$ and workup in H_2O gave **8**. Treatment of **8** with $Ph_3P{\cdot}HBr$ resulted in the salt **9**. The acetate[9] **10** was obtained by reaction of **9** with 4-acetoxy-2-methylcrotonaldehyde in the presence of BuLi at −50 °C. Saponification and oxidation of **10** gave a mixture of isomers (**11**).[7] After purification by column chromatography, the mixture was separated by high-pressure LC into the all-trans, 9-cis, 11-cis, and 13-cis isomers. Mass spectral analysis showed that the deuterium incorporation was ≥96% for 11,12-dideuterioretinal and ≥82% for 10-monodeuterioretinal. The analogue pigments were regenerated and purified, and Raman spectra were obtained by following previously published procedures.[4]

Figure 1A shows the RR spectrum of the photostationary steady-state mixture of unmodified pigments generated by 488-nm laser excitation at 77 K.[2] Lines near 854, 875, and 922 cm^{-1} are known to be exclusively due to bathorhodopsin.[1,4] Figure 1B displays the RR spectrum of the 10-monodeuterio steady state under the same conditions. The batho line at 875 cm^{-1} disappears while intensity increases at 707 and 829 cm^{-1}. The batho lines near 854 and 922 cm^{-1} are not shifted by this substitution. Figure 1C shows the RR spectrum of the 11,12-dideuterio steady state. The batho scattering at 922 cm^{-1} disappears while a new line appears at 683 cm^{-1} and intensity increases at 748 cm^{-1}. The frequencies of the batho lines at 875 and 854 cm^{-1} are only slightly shifted by this substitution.

Scheme I. Synthesis of 11,12-Dideuterioretinal (**6**) and 10-Monodeuterioretinal (**11**)[a]



[a] (a) $BrCH_2C{\equiv}CH$, Mg, $HgCl_2$, Et_2O; (b) $(CH_3CO)_2O$, 4-dimethylaminopyridine, toluene; (c) $NaNH_2$/DBN, toluene, reflux; (d) $CH_3COCH_2CH(OCH_3)_2$, BuLi, THF, −60 °C; (e) $LiAlD_4$, Et_2O, D_2O; (f) H_3PO_4 (85%), Et_2O; (g) CH_3CH_2Br, Mg, THF, $HC{\equiv}CH$; (h) $LiAlD_4$, Et_2O, H_2O; (i) $(C_6H_5)_3P{\cdot}HBr$, CH_3OH; (j) $CHOC(CH_3){=}CHCH_2OAc$, BuLi, THF, −50 °C; (k) 10% KOH in CH_3OH; (l) MnO_2, CH_2Cl_2.

The spectra in Figure 1 permit us to definitively assign the 875-cm^{-1} batho line to the $C_{10}H$ HOOP wag and the 922 cm^{-1} line to an $HC_{11}{=}C_{12}H$ HOOP mode.[10] The large observed deuterium shifts (875 → 707 cm^{-1}, 922 → 683 cm^{-1}) confirm that these modes involve predominantly hydrogen motion. These HOOP modes appear to be uncoupled from one another since the deuterium shift of one does not significantly perturb the frequencies of the remaining lines. This assignment for the anomalous bathorhodopsin vibrations supports our previous interpretation of the bathorhodopsin Raman spectrum[4] and the conclusion that the chromophore configuration is perturbed all-trans.[4,11]

This assignment to *specific* vibrational modes enables us to computationally model localized perturbations of the chromophore which can account for the batho vibrational frequencies and intensities. The batho 922-cm^{-1} $HC_{11}{=}C_{12}H$ HOOP is particularly important because this frequency is substantially below the

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[7] ^1H NMR ($CDCl_3$) of *all-trans*-11,12-dideuterioretinal: δ 10.11 (H_{15}, d), 6.31 (H_7, AB), 6.14 (H_8, AB), 6.18 (H_{10}, s), 5.96 (H_{14}, d), 2.33 (3 H_{20}, d), 2.03 (3 H_{19}, d), 1.72 (3 H_{18}, s), 1.9 (2 H_4, br m), 1.58–1.43 (2 H_2, 2 H_3, m), 1.04 (3 H_{16}, 3 H_{17}, s). ^1H NMR ($CDCl_3$) of *all-trans*-10-monodeuterioretinal: δ 10.11 (H_{15}, d), 7.14 (H_{11}, d), 6.36 (H_{12}, d), 6.33 (H_7, AB), 6.17 (H_8, AB), 5.97 (H_{14}, d), 2.33 (3 H_{20}, s), 2.03 (3 H_{19}, s), 1.9 (2 H_4, br m), 1.72 (3 H_{18}, s), 1.56–1.43 (2 H_2, 2 H_3, m), 1.04 (3 H_{16}, 3 H_{17}, s).

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[10] On the basis of our calculations,[4] the remaining intense bathorhodopsin line at 854 cm^{-1} must be assigned to either the $C_{14}H$ HOOP or the $HC_7{-}C_8H$ B_g HOOP. The proximity of the 854- and 875-cm^{-1} lines suggests that the corresponding HOOPs are localized at similar positions trans to methyl groups. We therefore attribute the 854-cm^{-1} line to the $C_{14}H$ HOOP.

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expected range (950–970 cm^{-1}) for this vibration.[12] In substituted ethylenes, this HOOP frequency can be significantly lowered by the inductive effect of groups covalently linked to the double-bonded carbons (e.g., 926 cm^{-1} in $HClC{=}CHCH_3$).[13] Our preliminary calculations show that this frequency can also be lowered by placing a negatively charged opsin residue near $C_{11}{=}C_{12}$ to reduce its bond order. A similar perturbation was recently suggested by Honig et al.[14] to explain the absorption spectra of rhodopsin analogues.

Whereas the frequencies of RR lines depend only on the ground-state conformation of the chromophore, their intensities also depend on the resonant excited-state geometry. The observed HOOP mode enhancement is a function of the displacement of the hydrogen equilibrium positions between the ground and excited states.[4,15] Thus, the fact that the 922-cm^{-1} batho line is enhanced means that the excited-state twist about the 11,12 double bond is different from that of the ground state, as intuitively expected for a cis → trans photoisomerization. The intensities of the 854- and 875-cm^{-1} HOOP modes can similarly be used to model relative twists about specific chain bonds in bathorhodopsin. This analysis should elucidate key features of the cis → trans isomerization trajectory in visual excitation.

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(12) The out-of-plane vibrations of hydrogens trans to a C=C double bond are coupled to form normal modes of approximately A_u and B_g local symmetry (C_{2h}). The higher frequency (A_u) vibration gives a reproducible IR group frequency between 950 and 970 cm^{-1} for a wide variety of model compounds, although it is normally silent in Raman spectra. Cf. ref 13. Even for the highly conjugated *all-trans*-retinal *n*-butylamine protonated Schiff base, the A_u HOOPs are found in the 950–970-cm^{-1} range (e.g., IR 967 cm^{-1}).

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Direct Measurement of the Electron Susceptibility Anisotropy in Paramagnetic Complexes Using High-Field Deuterium NMR

Sir:

A well-defined separation of the individual contact and pseudocontact contributions to the ^1H NMR chemical shifts of paramagnetic complexes requires an accurate knowledge of the elements of the susceptibility tensor ($\mathbf{x}$). The total shift is expressed as a sum of contact and dipolar terms, where the equation for the dipolar shift[1] (eq 1) contains the χ_{ii} values.

$$(\Delta H/H)_D = -\frac{1}{3}\left[\chi_{zz} - \frac{1}{2}(\chi_{xx} + \chi_{yy})\right]\langle(3\cos^2\theta' - 1)/R^3\rangle - \frac{1}{2}[\chi_{xx} - \chi_{yy}]\langle\sin^2\theta'\cos 2\varphi'/R^3\rangle \quad (1)$$

We report a direct measurement of the χ_{ii} on the basis of the observation of line splittings in deuterium NMR spectra. This solution-state NMR measurement eliminates extrapolation from low-temperature,[2] solid-state,[3] measurements of $\mathbf{x}$.

Placing a molecule with an anisotropic magnetic susceptibility in a strong magnetic field causes a partial cancellation of the random Brownian motion, and a small net alignment results. This partial order produces a nonzero average of anisotropic interactions; the quadrupole coupling energy of nucleii with $I \geq 1$ does not average to zero and leads to a splitting of absorption lines.

Here, we present observations of alignment effects in the high-resolution deuterium NMR spectrum of the paramagnetic, trigonal bis[phenyl-d_5-tris(1-pyrazolyl)borato]cobalt(II) system,[1b,4] abbreviated as Co(PTPB-d_5)$_2$, and we will show how these measurements directly yield the χ_{ii} necessary to quantify the pseudocontact shifts in eq 1. Data for the corresponding *p*-tolyl-d_7 derivative, depicted as Co(TTPB-d_7)$_2$, are also presented.

The room-temperature deuterium NMR spectra of Co(PTPB-d_5)$_2$ at three different field strengths (Figure 1) exemplify the results. The 21.14-kG spectrum clearly shows the three expected resonances with the para resonance substantially broader than the ortho and meta positions. Higher field data, at 46.98 kG, show the para resonance split, and the highest field spectrum (84.6 kG) exhibits additional splitting of the meta resonance and broadening of the ortho line. The field dependence of the para-deuteron splitting is included in Figure 2 as well as the temperature dependence of the *p*-CD_3 splitting in Co(TTPB-d_7)$_2$. The observed splittings are independent of concentration.

The alignment of any molecule in a magnetic field can be described by two order parameters[5]

$$S_0 = \langle(3\cos^2\theta - 1)/2\rangle = [\chi_{zz} - (\chi_{xx} + \chi_{yy})/2]H_0^2/15kT \quad (2a)$$

$$S_1 = \langle\sin^2\theta\cos 2\varphi\rangle = [\chi_{xx} - \chi_{yy}]H_0^2/15kT \quad (2b)$$

where θ and φ are the polar angles describing the H_0 vector relative to the susceptibility axes (x, y, and z). Magnetic alignment of an $I = 1$ quadrupole with an axially symmetric field gradient produces a residual quadrupole splitting[6]

$$\Delta\nu_Q = [(e^2qQ/h)H_0^2/20kT]\{(3\cos^2\alpha - 1) \times [\chi_{zz} - (\chi_{xx} + \chi_{yy})/2] + [(3\sin^2\alpha\cos 2\beta)/2](\chi_{xx} - \chi_{yy})\} \quad (3)$$

where α and β are the polar angles describing the C–D director relative to the magnetic axes. Since the combinations of the χ_{ii} in eq 3 are precisely those needed in eq 1, measured splittings of deuterium resonances with known α and β directly yield the magnetic anisotropy information for evaluation of dipolar shifts.

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