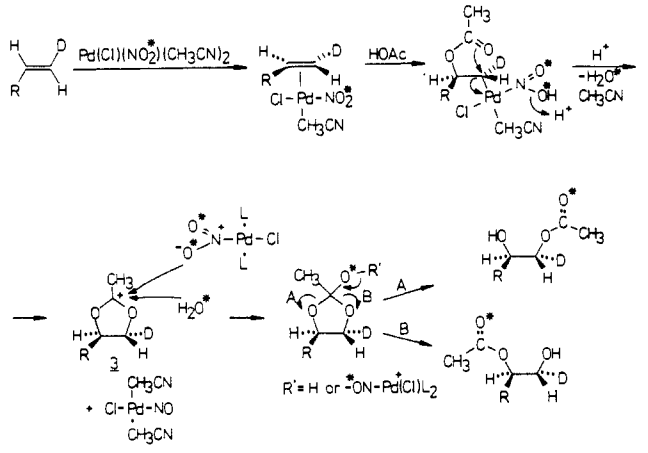
Scheme II



70/30) and subsequent ^{1}H NMR analysis showed that **1** and **2** are mainly of the threo configuration, the threo/erythro ratio being 88:12 in both cases. The configuration of **1** and **2** was established by ^{1}H NMR[12] and confirmed by hydrolysis to the diol, of which an independent sample of the erythro isomer was prepared.[13]

Since isomerization of olefin could be responsible for the loss of stereospecificity, the reaction was repeated with a 4-fold excess of (*E*)-1-deuterio-1-decene and was run for only 1 h. This led to only a slight improvement of the threo/erythro ratio to 91:9 (for both **1** and **2**), suggesting that isomerization of the olefin cannot account for all of the loss of stereospecificity. In accordance, ^{1}H NMR analysis of the recovered 1-deuterio-1-decene showed an *E*/*Z* ratio of 94:6. With a change of the *E*/*Z* ratio from 100:0 to 94:6 during the course of the reaction, we conclude that isomerization of the olefin can account for approximately one-third of the erythro isomer found in both cases.

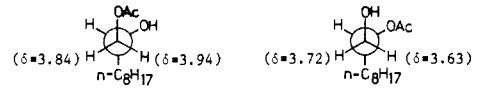
The stereochemical results for the oxidation of 1-deuterio-1-decene with $Pd(Cl)(NO_2)(CH_3CN)_2$[14] require that the palladium–carbon bond is cleaved with >94% inversion of configuration at carbon, and hence the mechanism suggested by Mares[4] cannot be correct. To account for both the stereochemical and labeling experiments, we suggest a mechanism via an acetoxonium intermediate (Scheme II). Trans acetoxypalladation followed by an oxidative cleavage of the palladium–carbon bond with inversion,[15] via neighboring group attack,[16] would give the five-membered cyclic cationic intermediate **3**. In this process, labeled water is generated.[17] The labeled water, or alternatively a coordinated O-labeled nitro group, may now attack the cyclic cationic intermediate at the carbonyl carbon followed by rearrangement to either **1** or **2**. This would explain the 1:1 ratio between **1** and **2**.[18] The 2-decanone can be formed either by the same mechanism as given by Mares[4] or via a Wacker reaction by the water released.

A related mechanism to that proposed here was suggested by Yermakov and co-workers[19] to account for the ^{17}O label in the carbonyl group of ethylene glycol monoacetate obtained from oxidation of ethene by $LiN^{17}O_3/Pd(OAc)_2$ in acetic acid. It is interesting to note that the mechanism proposed in Scheme II has similarities with the mechanism of the "wet" Prevost reaction for preparation of glycol monoacetate from olefins with overall cis addition using bromine and silver acetate in acetic acid.[20]

We conclude that the mechanism proposed here is consistent with stereochemical data, labeling studies, and the regiochemistry observed. In the oxidation of internal olefins to glycol monoacetates by the system $O_2/Pd^{II}/LiNO_3/LiCl$ in acetic acid, an overall cis addition of OH and OAc has been reported.[21] However, the addition to terminal olefins was regioselective and a different mechanism to that reported here is probably operating.

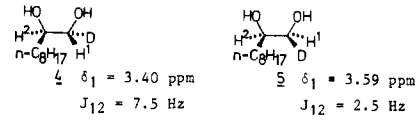
Acknowledgment. We thank the Swedish Natural Science Research Council and CNRS for financial support.

(12) The ^{1}H NMR spectra of the undeuterated parent compounds: **1-acetoxy-2-decanol** ($CDCl_3$) δ 4.14 (dd, J = 11.0, 2.7 Hz, 1 H, CH_2OAc), 3.94 (dd, J = 11.0, 7.4 Hz, 1 H, CH_2OAc), 3.84 (m, 1 H, $CHOH$), 2.10 (s, 3 H, OAc), 1.46 (m, 2 H), 1.28 (m, 12 H), 0.88 (br t, 3 H); **2-acetoxy-1-decanol** ($CDCl_3$) δ 4.91 (m, 1 H, $CHOAc$), 3.72 (dd, J = 12.0, 2.9 Hz, 1 H, CH_2OH), 3.63 (dd, J = 12.0, 6.2 Hz, 1 H, CH_2OH), 2.09 (s, 3 H, OAc), 1.58 (m, 2 H), 1.28 (m, 12 H), 0.88 (br t, 3 H). Since gauche conformations between the hydroxy and acetoxy groups are preferred, the following assingments of the terminal diastereotopic methylene protons can be made:



The product **1** showed a doublet (J = 7.6 Hz) at δ 3.94 and the product **2** showed a broad doublet (J = 6.3 Hz) at δ 3.61.

(13) Hydrolysis of **1** and **2** (NaOH, EtOH–H_2O) in both cases gave *threo*-1-deuterio-1-decane-1,2-diol (**4**). A reference sample of the erythro isomer **5** was prepared by epoxidation of (*E*)-1-deuterio-1-decene and subsequent acid-catalyzed ring opening in aqueous THF. The ^{1}H NMR ($CDCl_3$ + D_2O) spectra of **4** and **5** are different.



(14) The analogous oxidation in the presence of LiOAc according to Mares[4] gave the same stereochemical result but with a slightly higher stereospecificity (threo:erythro = 95:5).

(15) Bäckvall, J. E. *Acc. Chem. Res.* **1983**, *16*, 335.

(16) Bäckvall, J. E.; Nordberg, R. E. *J. Am. Chem. Soc.* **1980**, *102*, 393.

(17) Previous studies show that the labeled water would not exchange significantly with acetic acid under the reaction conditions. Oxygen atom exchange of acetic acid with water is slow and requires the presence of strong mineral acids to occur at a reasonable rate: Bentley, R. *J. Am. Chem. Soc.* **1949**, *71*, 2765. Llewellyn, D. R.; O'Connor, C. *J. Chem. Soc.* **1964**, 545. O'Connor, C.; Turney, T. A. *J. Chem. Soc. B* **1966**, 1211.

(18) The mechanism in Scheme II would seem to give ≤100% yield of oxidation products based on palladium, which is at variance with experimental results.[4] However, the final $PdCl(NO)(CH_3CN)_2$ may continue to react to give Wacker-type oxidation products and Pd(O).

(19) Kuznetsova, N. I.; Likholobov, V. A.; Fedotov, M. A.; Yermakov, Y. I. *J. Chem. Soc., Chem. Commun.* **1982**, 973.

(20) Wiberg, K. B.; Saegebarth, K. A. *J. Am. Chem. Soc.* **1957**, *79*, 6256.

(21) (a) Yoshimura, N.; Tamura, M. *Abstr. Int. Conf. Organomet. Chem., 8th* Kyoto, Japan, 1977; 3Co7, p. 251. (b) Yoshimura, N. *Jpn. Kokai Tokkyo Kono* 76 08, 210; *Chem. Abstr.* **1976**, *85*, 32441r. (c) Tamura, M.; Yasui, T. *J. Chem. Soc. D* **1968**, 1209.

Identification of the Altered Pyrrole in Sulfmyoglobin and an Extractable "Sulfhemin": Participation of the 4-Vinyl Group in the Saturation of the Pyrrole in One Form of Sulfmyoglobin

Mariann J. Chatfield, Gerd N. La Mar,*
Juliette T. J. Lecomte, Alan L. Balch, Kevin M. Smith, and Kevin C. Langry

Department of Chemistry, University of California Davis, California 95616

Received May 9, 1986

The sulfglobins are nonfunctional forms of either myoglobin or hemoglobin where the sequential reaction of the reduced proteins with an oxidizing agent and thiol leads to the formation of a green pigment.[1] The optical spectra have indicated the reduction of a pyrrole to yield a chlorin-type macrocycle which ^{35}S tracer work has shown to contain one atom of sulfur per heme, frequently envisaged as an episulfide across the pyrrole β positions.[2] To date neither the identity of the modified ring nor the chemical nature of the reacted site(s) has been elucidated. While recent spectroscopic studies aimed at elucidating the structure of SMb agree on evidence of a strongly perturbed tetrapyrrole, there is

(1) Berzofsky, J. A.; Peisach, J.; Blumberg, W. E. *J. Biol. Chem.* **1971**, *246*, 3367–3377.

(2) Berzofsky, J. A.; Peisach, J.; Horecker, B. L. *J. Biol. Chem.* **1972**, *247*, 3783–3791.

0002-7863/86/1508-7108$01.50/0

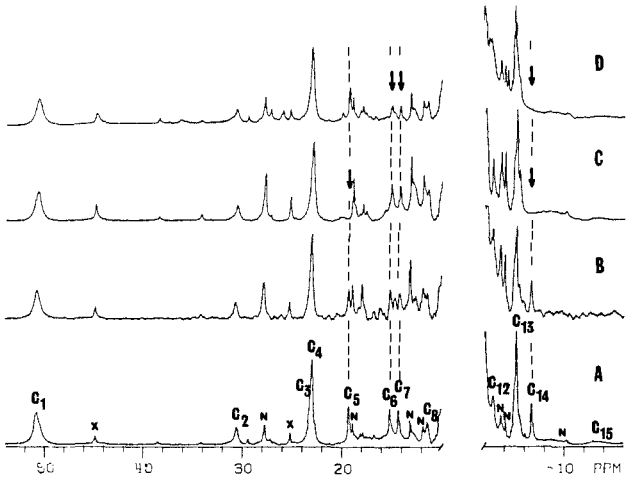


Figure 1. Resolved portions of the 360-MHz ^1H spectra of S_CMbCN at 20 °C in 0.1 M phosphate buffer in ^2H_2O, pH 7. (A) Sample prepared as described previously;[5] S_CMb peaks are labeled C_i. Peaks due to residual native protein and other forms of SMb are labeled N and X, respectively. (B) Sample prepared by reconstituting fresh apoMb with the green sulfhemin extracted from sample presented in (A) and adding CN^-. (C, D) samples prepared as in (A) by using Mb reconstituted with [2,4-($^2H_\alpha$)$_2$]hemin (>90% deuterated at both sites[9]) and [4,4,4-$^2H_3$]hemin (>90% $^2H_\alpha$, ~30–40% ($^2H_\beta$)$_2$), respectively. Reduced peak intensities due to deuteration are indicated by arrows.

lack of agreement even as to the involvement of vinyls in SMb formation.[3,4] We have demonstrated recently that ^1H NMR spectroscopy can detect three distinct forms (designated S_AMb, S_BMb, and S_CMb in order of appearance) of SMb prepared by literature methods.[5] The three species cannot be distinguished by optical spectroscopy but provide clearly different NMR spectra, particularly as metcyano complexes. Some of the conditions that favor the formation of the individual species have also been characterized.[5]

We demonstrate herein that one of the three characterized forms of sperm whale sulfmyoglobin, S_CMb, allows extraction of a green "sulfhemin" pigment that is stable for many hours at ambient temperatures. The original S_CMb can be reconstituted from this "sulfhemin" and apoMb. Isotope labeling of the vinyl positions unambiguously establishes that the 4-vinyl group has reacted in both the extracted green pigment and S_CMb. The 360-MHz ^1H NMR spectrum of the metcyano complex of the C form of sulfmyoglobin, metS_CMbCN, prepared as described previously,[5] is illustrated in Figure 1A. Extraction of the $S_C$hemin by the method of Teale,[5,6] followed by reconstitution into fresh apoMb,[7] yields the trace in Figure 1B; while some $S_C$hemin has reverted back to hemin, yielding native disordered[8] metMbCN, the other signals are the same as those of metS_CMbCN (Figure 1A). Thus the reduced chromophore appears unaltered by the extraction procedure. The ^1H NMR traces of metS_CMbCN reconstituted with [2,4-($^2H_\alpha$)$_2$]hemin[9] and [4,4,4-$^2H_3$]hemin[9] are shown in C and D of Figure 1; trace C identifies C_{14} as 4-H_α and C_6 and C_7 as the two 4-H_βs. The 2-vinyl H_α is designated as C_5.

Dissolution of the extracted $S_C$hemin in Me_2SO-d_6 in the presence of an excess of CN^- yields the 500-MHz ^1H NMR trace shown in Figure 2A; the peaks marked P arise from the dicyano complex of both unreacted and regenerated hemin.[10] The peaks

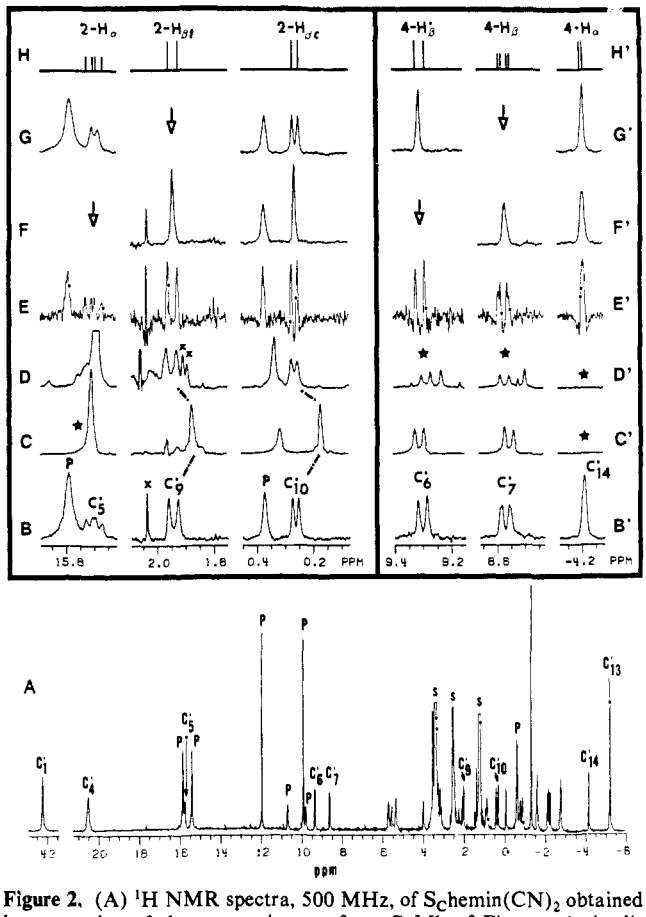


Figure 2. (A) ^1H NMR spectra, 500 MHz, of $S_C$hemin(CN)$_2$ obtained by extraction of the green pigment from S_CMb of Figure 1A, in dimethyl-d_6 sulfoxide at 30 °C. Present are $S_C$hemin(CN)$_2$ (peaks labeled C'_1), as well as residual and regenerated hemin(CN)$_2$ (peaks labeled P). X and S represent impurities and solvents Expanded regions of the sulfhemin(CN)$_2$ spectrum relevant to the assignment of the 2- and 4-resonances are shown in (B) and (B'), respectively. (C, C') These expanded regions for [2,4-($^2H_\alpha$)$_2$]sulfhemin(CN)$_2$ extracted from the sample used to obtain Figure 1C; note the loss (marked by a star) of peaks C'_5 and C'_{14} (H_αs) and the collapse of the doublet structures for C'_9 and C'_{10} (peaks positions are slightly variable due to aggregation effects[10]). (D, D') Expanded regions for the [4,4,4-$^2H_3$]sulfhemin(CN)$_2$ extracted from the sample used to obtain Figure 1D; note the reduced intensities (marked by a star) solely for C'_6, C'_7, and C'_{14}. The complex structures of C'_6 and C'_7 are due to partial deuteration of the β-positions and isotope effects on the hyperfine shift.[14] (E, E') Spectra of (B, B') after resolution enhancement, revealing additional couplings for peaks C'_7 and C'_{14}. (F, G), (F', G') Effect of decouplings within the 2-vinyl and the "4-vinyl" protons, with the decoupler frequency marked by an arrow. The resolved multiplet structures deduced from the decoupling results, together with the proton labeling, are schematically represented in (H) and (H') for the 2- and 4-substituents, respectively.

marked C' arise from $S_C$hemin(CN)$_2$; three methyl peaks, C'_1, C'_4, and C'_{13} resonate at positions very similar to those of the apparent heme methyls in metS_CMbCN (Figure 1A). Spectral regions relevant to the 2- and 4-substituent assignments are expanded in Figure 2, parts B and B', respectively. Extraction of [2,4-($^2H_\alpha$)$_2$]$S_C$hemin and [4,4,4-$^2H_3$]$S_C$hemin yields samples whose spectra differ from that of $S_C$hemin(CN)$_2$ in the reduced intensities of two and three C' resonances, respectively, as shown in Figure 2C,C',D. Thus peak C'_5 arises from the 2-H_α, C'_{14} from the 4-H_α, and C'_6, C'_7 from the 4-H_βs. Decoupling of 2-H_α (Figure 2F) collapses the structure of C'_9 and C'_{10}, which exhibit 11- and 17-Hz couplings to 2-H_α, respectively. Furthermore, irradiation of the C'_9 transitions (Figure 2G) slightly sharpens the C'_{10} signal;

(3) Anderson, L. A.; Loehr, T. M.; Lim, A. R.; Mauk, A. G. *J. Biol. Chem.* **1984**, *259*, 15340–15349.
(4) Timkovich, R.; Vavra, M. R. *Biochemistry* **1985**, *24*, 5189–5196.
(5) Chatfield, M. J.; La Mar, G. N.; Balch, A. J.; Lecomte, J. T. J. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 309–315.
(6) Teale, F. W. J. *Biochim. Biophys. Acta* **1959**, *35*, 543.
(7) La Mar, G. N.; Toi, H.; Krishnamoorthi, R. *J. Am. Chem. Soc.* **1984**, *106*, 6395–6401.
(8) La Mar, G. N.; Davis, N. C.; Parish, D. W.; Smith, K. M. *J. Mol. Biol.* **1983**, *168*, 887–896.
(9) Smith, K. M.; Fujinari, E. M.; Langry, K. C.; Parish, D. W.; Tabba, H. D. *J. Am. Chem. Soc.* **1983**, *105*, 6638–6646.

(10) The chemical shifts of the resonances were found to be variable with concentration and solvent composition, which is consistent with aggregation (Viscio, D. B.; La Mar, G. N. *J. Am. Chem. Soc.* **1978**, *100*, 8096–8100 and references therein).

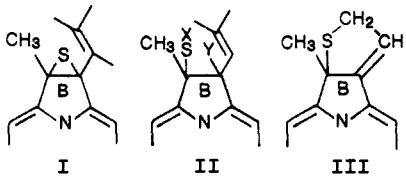
therefore C'_9 and C'_{10} originate from the 2-$H_{\beta-c}$ and 2-$H_{\beta-t}$ of an unmodified vinyl group.[11]

In contrast with the native hemin vinyls and the $S_C$hemin 2-vinyl, the three protons from the original 4-vinyl group do not exhibit the characteristic 11- and 17-Hz coupling constants.[11] Deconvolution (Figure 2E') and spin-system simulation indicate for peak C'_{14} couplings of ~1 and 3.6 Hz. This latter coupling is removed upon on-acquisition irradiation of C'_7 (Figure 2G'). The remaining 15-Hz splitting of C'_6 and C'_7 disappears from either peak by irradiating the other (Figure 2F',G'); this large coupling between the two 4-H_βs corresponds well to the geminal coupling of a saturated methylene pair.[12] C'_6, C'_7, and C'_{14} present no other detectable coupling (≥1 Hz) whether the reaction is carried out completely in H_2O or ^2H_2O. Consequently, the coupling pattern of the 4-substituent dictates that the $C_\alpha H$–$C_\beta H_2$ fragment no longer contains the double bond and did not undergo addition of any proton.

Since the ^{1}H NMR spectra of the three previously reported metSMbCN complexes are very similar, we expect that the same pyrrole is reduced in each derivative, with only differing substituent functionalities. The similar resonance positions of the original 4-H_α and 4-H_βs and of the resolved methyls in the extracted pigment (Figure 2A,B) and metS_CMbCN (Figure 1A) argue strongly for identical structures of the modified hemin. *Thus the saturated ring in sulfmyoglobin is pyrrole B.* This conclusion differs from the proposed site of attack presented in recent studies.[3,4]

This constitutes clear evidence for vinyl group participation in the formation of *one* of the forms of SMb, namely, S_CMb. On the other hand, since sulfmyoglobin complexes analogous to S_AMb and S_BMb have been detected by ^{1}H NMR for Mb reconstituted with deuteroheme[13] (2,4-vinyls replaced by hydrogens), it is obvious that *vinyls are not necessary to the formation of the initial SMb complex.* The direct participation of vinyl groups in SMb formation in one of the three forms may account for the difficulty in establishing the presence of intact vinyls in SMb preparations using resonance Raman spectroscopy.[3] The simple reaction of the 4-vinyl group to form an episulfide (or epoxide) can be discounted since such strained cyclic systems exhibit much smaller geminal couplings (5–7 Hz).[12]

The multiplet structure for the protons of the reacted 4-vinyl group, however, is completely consistent with the formation of a cyclic thioether, as depicted in III below; this is equivalent to



I II III

the reduction of the buried B pyrrole, whereas others suggested reduction of ring A or less probably B,[3] or C or D.[4] The observed coupling constants are very close to those of the similarly structured 3-thiolenes.[12] The mechanism of formation of III (S_CMb) could involve the process I → III, with the presumed initially formed episulfide[2] (I) reacting with the 4-vinyl C_β. An alternate pathway would have II as the precursor, which, in turn, could be formed from I via attack of a protein-based or exogenous nucleophile. Thus we tentatively suggest the pathways $\overrightarrow{I \rightarrow II \rightarrow III}$ as representing the reaction sequences $\overrightarrow{S_AMb \rightarrow S_BMb \rightarrow S_CMb}$.

Acknowledgment. This research was supported by grants from the National Institutes of Health, HL 16087, 22252, and 26226.

(11) Scheer, H.; Katz, J. J. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; pp 412–416.
(12) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969; Vol. 5, pp 270–328 and references therein.
(13) Chatfield, M. J.; La Mar, G. N.; Balch, A. L.; Smith, K. M.; Parish, D. W.; LePage, T. J. *FEBS Lett.*, in press.
(14) La Mar, G. N.; Smith, K. M.; Gersonde, K.; Sick, H.; Overkamp, M. *J. Biol. Chem.* **1980**, *255*, 66–70.

Fourier Transform Infrared Vibrational Circular Dichroism of Matrix-Isolated Molecules

D. O. Henderson and P. L. Polavarapu*

Department of Chemistry, Vanderbilt University Nashville, Tennessee 37235

Received April 28, 1986

Vibrational absorption measurements on matrix-isolated molecules have proved to be important with exciting applications[1] such as mode-selective excitation of reactions. Vibrational circular dichroism (VCD), which is a measure of differential absorption of left vs. right circularly polarized infrared radiation and was successfully measured,[2] has unique potential for revealing the three-dimensional structure of chiral molecules. Noting the numerous advantages in combining the VCD and matrix-isolation techniques, Schlosser et al.[3] demonstrated the feasibility of VCD measurements on α-pinene and β-pinene in Ar matrices at 4-cm^{-1} resolution in the 3100–2800-cm^{-1} region. For these measurements, a dispersive spectrometer and slow spray-on (SSO) procedure, with deposition times of 2–4 h, were used. Although FTIR instruments have been successfully used[4,5] for VCD measurements on neat liquids, their use for VCD measurements on matrix-isolated molecules remains to be established, especially since the procedures of and artifacts in FTIR and dispersive VCD techniques are different.

For several organic molecules, α-pinene for example, the absorptions of the bands in the 1600–800-cm^{-1} region are 1–2 orders of magnitude smaller than those of the intense bands in the 3100–2800-cm^{-1} region. Then for VCD measurements in the 1600–800-cm^{-1} region SSO deposition would require prohibitively long periods of time and introduce scattering and leak problems, there by questioning the feasibility of such measurements. In this paper, using pulsed matrix isolation[6] (PMI) we demonstrate that FTIR-VCD measurements are not only feasible in the 1600–800-cm^{-1} region but also obtainable at a higher resolution (1 cm^{-1}) than was achievable to date. FTIR-VCD of unusual molecules synthesized and (or) stable only at low temperatures may thus become feasible.

A closed-cycle cryostat (Air products) was inserted into the sample compartment of an FTIR spectrometer (Nicolet 6000C) without physical contact between the two. Nitrogen–pinene mixtures premixed in a 2000-mL bulb (~600 torr of nitrogen was bled into the bulb saturated with pinene vapor at ~2 torr) are deposited on to KBr window at ~15 K through a 45° port in pulses, each pulse containing ~25 mL of mixture at about 600 torr. The open end of transfer line, which was made of copper with ~0.8-mm inner diameter, was located at about 2 cm from the deposition window. The deposition time is usually less than $^1/_2$ h. Transparent matrices with matching base lines for the enantiomers were obtained. Deposition of one or two pulses of matrix gas, prior to the mixture, significantly reduced the light scattering. Nitrogen matrices were found to be more transparent than argon matrices. An optical filter with 5% cut-on at 1650 cm^{-1}, BaF_2 polarizer, and ZnSe photoelastic modulator, with modulation frequency (ω_m) of 97 KHz, preceded the sample. A HgCdTe detector and lock-in amplifier were used[4,5] for signal processing. Six data files, each containing 1000 coadded ω_m interferograms and another six data files each containing 50 coadded transmission interferograms, were collected. For each interferogram 8192 data points were collected with one data point

(1) Frei, H.; Pimentel, G. C. *Annu. Rev. Phys. Chem.* **1985**, *36*, 491–524.
(2) Holzwarth, G.; Hsu, E. C.; Mosher, H. S.; Faulkner, T. R.; Moscowitz, A. *J. Am. Chem. Soc.* **1974**, *96*, 251–252. Nafie, L. A.; Keiderling, T. A.; Stephens, P. J. *J. Am. Chem. Soc.* **1976**, *98*, 2715–2723.
(3) Schlosser, D. W.; Devlin, F.; Jalkanen, K.; Stephens, P. J. *Chem. Phys. Lett.* **1982**, *88*, 286–291.
(4) Nafie, L. A.; Diem, M.; Vidrine, D. W. *J. Am. Chem. Soc.* **1979**, *101*, 496–498. Nafie, L. A.; Vidrine, D. W. *Fourier Transform Infrared Spectrosc.* **1982**, *3*, 83.
(5) Polavarapu, P. L. *Fourier Transform Infrared Spectrosc.* **1985**, *4*, 61.
(6) Rochkind, M. M. *Anal. Chem.* **1967**, *39*, 567–574. Perutz, R. N.; Turner, J. J. *J. Chem. Soc., Faraday Trans. 2* **1973**, *69*, 452–461.

0002-7863/86/1508-7110$01.50/0