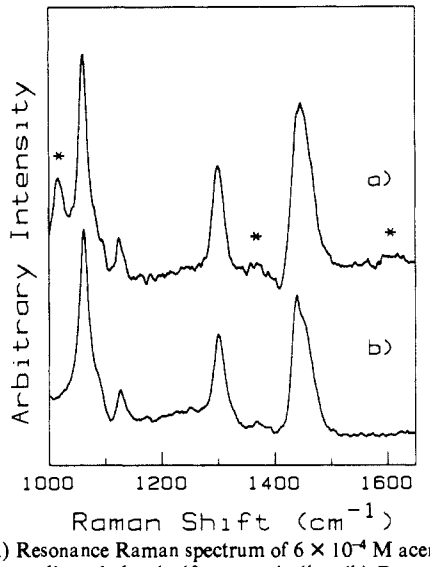

Figure 2. (a) Resonance Raman spectrum of 6×10^{-4} M acenaphthylene in a thallium-sodium dodecylsulfate co-micelle. (b) Raman spectrum of micellar solution blank. Conditions are the same as in Figure 1. Bands attributable to acenaphthylene are denoted by *.

In this work, we report a new approach to resonance Raman spectroscopy which makes use of specially functionalized micelles.[28] It is thought that this micellar method will make RRS a relatively sensitive and widely applicable technique in which an analyte's luminescent properties are no longer of critical importance. There are several ways functionalized micelles can be used to advantage in RRS. First, micelles can shift both absorption and luminescent band maxima.[29,30] Shifting the absorption band closer to the laser excitation line gives one a greater resonance effect. For example, the Soret band of methanolic zinc tetraphenylporphyrin tetrasulfonic acid (used in this study) undergoes a bathochromic shift of 6 nm when in aqueous micellar solution. Even this modest shift resulted in a 2-fold change in the extinction coefficient at a fixed wavelength. Spectral shifts an order of magnitude higher have been reported. Shifting the background luminescence away from the Raman bands is equally advantageous. Micelles can also alter an associated compound's luminescent lifetime and quantum yield.[29,30] In RRS it is desirable to significantly decrease both of these. This is accomplished by utilizing surfactants containing heavy atoms and/or free radicals. This quenching effect is shown in Table I. Micelles also can enhance nonradiative processes by bringing a luminescent compound into close contact with other added quencher molecules. Mechanistically, this process is somewhat akin to that of micellar inhibition and catalysis.[31] Micelles allow solubilization of hydrophobic solutes in water (a good Raman solvent) as opposed to organic solvents which produce strong, interfering Raman bands. Indeed, the signal to noise ratio can be increased up to 2 orders of magnitude in aqueous micellar solution. This was first demonstrated by Beck and Brus for transient spontaneous Raman scattering.[32] Micelles also can stabilize transient species that are difficult to observe in other solvents (e.g., radical ions), as well as preventing photodecomposition and altering the fluorescence depolarization and pH profile of a variety of compounds.[29,30]

Judicious use of one or more of the aforementioned micellar effects allows one to record resonance Raman spectra that were previously difficult or impossible to obtain. Figure 1 shows the first reported resonance Raman spectrum (to our knowledge) of a 4.3×10^{-5} M solution of zinc tetraphenylporphyrin tetrasulfonic acid ($ZnTTPS_4$) obtained with direct Soret excitation.[33] No evidence of photodecomposition (a common problem with tetraphenylporphyrins upon laser irradiation) was observed in the absorption spectra of samples prepared in Brij "cocktail" (see Table I for the exact composition of the micellar solutions). The prominent ν_4 band at 1360 cm^{-1} and ν_2 and ν_{11} bands at ~1586 nm, as well as a number of weaker bands, are easily observable. The background from the micellized surfactant is minimal (Figure 1). Figure 2 shows the resonance Raman spectrum of 6×10^{-4} M acenaphthylene in another micellar cocktail.[33] Characteristic bands are noted at 1020 cm^{-1} and above 1580 cm^{-1}. Although the surfactant bands are more prominent in this spectrum, note that the surfactant is several orders of magnitude more concentrated than the acenaphthylene.

The use of functionalized micelles in RRS is simple and highly advantageous. A knowledge of the physical and chemical properties of micelles[34] is beneficial in that it allows one to choose the optimum combination of effects to enhance RRS. It is hoped that this will make RRS an even more widely applicable technique for the study of molecular structure. It may also allow the first effective use of Raman in hyphenated techniques such as LC-resonance Raman.

Acknowledgment. The support of this work by the Department of Energy, Office of Basic Energy Science (DE-AS0584ER13159), is gratefully acknowledge by D.W.A. and that of the N.I.H. (GM33330) by M.R.O.

(28) Functionalized micelles contain surfactants with specific functional groups. The purpose of the functional group is to enhance the desired micellar effect. For example, a surfactant containing a heavy atom will tend to enhance fluorescence quenching of certain compounds.

(29) Singh, H. N.; Hinze, W. L. *Analyst (London)* **1982**, *107*, 1073.

(30) Hinze, W. L.; Singh, H. N.; Baba, Y.; Harvey, N. G. *Trends Anal. Chem.* **1984**, *3*, 193.

(31) Fendler, J. H. *Membrane Mimetric Chemistry*; Wiley: New York, 1982.

(32) Beck, S. M.; Brus, L. E. *J. Chem. Phys.* **1981**, *75*, 1031.

(33) A Molectron UV-24 nitrogen laser is used to pump a Molectron DL-II dye laser. A backscattering geometry is used to illuminate the sample. The scattered radiation is collected and passed through a polarization scrambler before being focused into a SPEX 1403 scanning monochromator. a photomultiplier (Hamamatsu R-928) in a cooled housing is used for signal detection. The output of the PMT is directed into a EG&G Model 162 boxcar with a Model 164 plug-in integrator card. The boxcar is triggered by a photodiode which is triggered by the laser pulse. The output of the boxcar is fed into a SPEX Datamate (DM-1), which controls the monochromator and stores the collected data. The data is then transferred via an RS-232 interface to an Apple II series computer for storage on a floppy disk and subsequent graphic manipulation. Excitation was at 402 nm, and the solvent was as indicated in the footnote of Table I.

(34) Armstrong, D. W. *Sep. Purif. Methods* **1985**, *14*, 213.

Ethylene Biosynthesis. 7. Secondary Isotope Effects

Michael C. Pirrung*[1] and Gerard M. McGeehan

Department of Chemistry, Stanford University
Stanford, California 94305

Received May 9, 1986

In a continuing investigation of the mechanism of the biosynthesis of ethylene, the plant ripening hormone, from 1-aminocyclopropanecarboxylic acid, the study of isotope effects was undertaken in order to provide insight into the rates of various bond-breaking steps. While the applicability of the concept of "rate-limiting step" to enzymatic reactions has been questioned[2] and redefined[3] (and there is little doubt an ethylene-forming enzyme exists[4]), such studies were expected to elucidate some mechanistic detail as well as measure the validity of a model for ethylene biosynthesis.[6]

(1) Research Fellow of the Alfred P. Sloan Foundation, 1986-1989.

(2) Northrop, D. B. *Biochemistry* **1981**, *20*, 4056.

(3) Ray, W. J. *Biochemistry* **1983**, *22*, 4625.

(4) For example, the stereoselectivity observed with alkyl-ACC analogues is difficult to rationalize otherwise. See: Hoffman, N. E.; Yang, S. F.; Ichihara, A.; Sakamura, S. *Plant Physiol.* **1982**, *70*, 195. Pirrung, M. C.; McGeehan, G. M. *J. Org. Chem.*, in press. Baldwin, J. E., Adlington, R. M., Lajoie, G. A.; Rawlings, B. J. *J. Chem. Soc., Chem. Commun.* **1985**, 1496. Furthermore, the ability to inactivate the ethylene-synthesizing system by a mechanism-based strategy also strongly suggests this conclusion.[5]

(5) Pirrung, M. C.; McGeehan, G. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *20*, 1044.

(6) Pirrung, M. C. *J. Am. Chem. Soc.* **1983**, *105*, 7207.

0002-7863/86/1508-5647$01.50/0

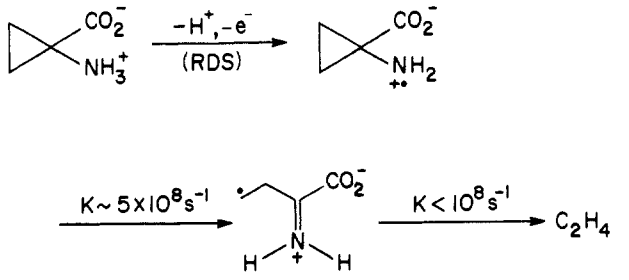

Figure 1.

Secondary isotope effects for the conversion of ACC and ACC-d_4[7] to C_2H_4/C_2D_4 by both mung bean hypocotyl segments and the electrochemical model[6] for ethylene biosynthesis were measured by the competition method. A 1:1[8] mixture of tetraprotio- and tetradeuterio-ACC was fed to mung bean.[9] After 72 h of feeding (<1% conversion), the head space was analyzed by GC/MS.[10] The observed ratio of isotopomers reveals $k_H/k_D = 0.990 \pm 0.014$.[11] Similarly, when the same mixture of tetraprotio- and tetradeuterio-ACC used above was converted to its tetrabutylammonium salt and oxidized at +0.8 V vs. SCE (<3% conversion) in CH_3CN, the observed isotope ratio reveals $k_H/k_D = 1.005 \pm 0.015$.

$$\text{ACC/ACC-}d_4 \xrightarrow{\text{mung bean}} \frac{C_2H_4}{C_2D_4} \Longleftrightarrow \frac{m/z = 27}{m/z = 30}$$

$$k_H/k_D = 0.990 \pm 0.014$$

The lack of an isotope effect for either oxidative conversion of ACC to ethylene when a significant effect might be expected[12] suggests that no step which would be isotope-sensitive contributes to the rate limitation. This is certainly true for the electrochemical oxidation, but because of the nature of competition experiments, only a V/K isotope effect is obtained from the in vivo experiment.[13] Such isotope effects do not reflect rate limitation, only the forward commitment for an isotope-sensitive step, which here must be great. This satisfies one's chemical intuition that once the cyclopropane ring has opened, conversion to products is highly favored over reclosure. This conclusion was also foreshadowed by data showing that *cis*-ACC-d_2 recovered from oxidations retains its stereochemistry.[14]

The inference that the initial one-electron oxidation of aminocyclopropanecarboxylic acid is rate limiting has precedent. Electrochemical and chemical oxidation of amines is known to have this characteristic.[15] In studying the mechanism-based inactivation of cytochrome P-450 by heteroatom-substituted cyclopropanes, Macdonald and Guengerich have found a linear correlation between the one-electron oxidation potential for the substrate and its log k_{inact}.[16] This implies that the rate-limiting step for inactivation events is the initial one-electron oxidation. The mechanistic similarities between inactivation of P-450, by cyclopropylamines, for example, and ethylene biosynthesis are striking.

The current data and recent studies on cyclopropyl-ACC[5] allow the proposal of a partial kinetic mechanism for ethylene biosynthesis (Figure 1). For the electrochemical model (and likely for the biosynthetic reaction, though no proof exists for this) the production of the amine radical cation is rate-limiting. Ring opening is assumed to occur at a rate similar to Ingold's measurements on cyclopropylaminyl radicals.[17] This ring-opened intermediate may not give nonradical products (i.e., ethylene) at a rate faster than that shown; otherwise, vinylcyclopropane would be expected as a product from cyclopropyl-ACC, a result contrary to fact in the biosynthetic and model[18] reactions. Other free-radical clocks may be used to more firmly define the lifetime of this intermediate.

Acknowledgment. Financial support from the U.S.-Israel Binational Agricultural Research & Development Fund (Grant I-643-83) is appreciated. M.C.P. is a Presidential Young Investigator (NSF CHE 84-51324).

(7) Prepared by applying a published procedure[6] to tetradeuteriodibromoethane.
(8) Determined by mass spectrometry of the methyl esters.
(9) Vegetative tissue such as mung bean does not have the capability to biosynthesize ACC in the absence of other phytohormones. Thus the only substrate present is what is provided. It was confirmed by adding compounds known to be inhibitors of ACC biosynthesis (e.g., aminooxyacetic acid) that no endogenous ethylene modified the isotope ratio.
(10) For a general description of the MS techniques used to analyze isotopically labeled gases, see: Pirrung, M. C. *Bioorg. Chem.* **1985**, *13*, 219. In this case, authentic C_2H_4/C_2D_4 mixtures were diluted to the same concentration of ethylene as in the sample and used for calibration. Isotope ratios were measured at the M - 1 ion of ethylene and M - 2 ion of perdeuterioethylene, obviating interference from N_2 and O_2.
(11) All errors given for these isotope effects refer to one standard deviation.
(12) The secondary isotope effect predicted (Streitweiser, A.; Jagow, R. H.; Fahey, R. C.; Suzuki, S. *J. Am. Chem. Soc.* **1968**, *80*, 2326) from IR data, using the stepwise mechanism previously proposed,[6] depends on which carbon-carbon bond cleavage is limiting. Radical additions to ethylene have $k_D/k_H \sim 1.1$ (Stefani, A. P.; Chuang, L. Y.; Todd, H. E. *J. Am. Chem. Soc.* **1970**, *92*, 4168). Olivella et al. (Olivella, S.; Canadell, E.; Poblet, J. M. *J. Org. Chem.* **1983**, *48*, 4696) have predicted isotope effects of approximately this magnitude. While ring opening is likely to have a very small isotope effect due to the countervailing influence of the hybridization changes at the two methylenes, this step is unlikely to be limiting. It is also important to note that good evidence exists that transport is not limiting in the processing of ACC analogues: Venis, M. A. *Planta* **1984**, *162*, 85.
(13) Northup, D. B. In *Isotope Effects on Enzyme-Catalyzed Reactions*; Cleland, W., O'Leary, M. H., Northrup, D. B., Eds.; University Park Press: Baltimore, 1977; p 122.
(14) Pirrung, M. C.; Yang, S. F., unpublished results.
(15) Hull, L. A.; Giordano, W.; Rosenblatt, D.; Davis, G.; Mann, C.; Milliken, S. *J. Phys. Chem.* **1969**, *73*, 2147.
(16) Guengerich, F. P.; Willard, R. J.; Shea, J. P.; Richards, L. E.; Macdonald, T. L. *J. Am. Chem. Soc.* **1984**, *106*, 6446.
(17) Griller, D.; Ingold, K. *Acc. Chem. Res.* **1980**, *13*, 317.
(18) McGeehan, G. M. Ph.D. Thesis, Stanford University, 1986.

Use of Chiral Single Crystals To Convert Achiral Reactants to Chiral Products in High Optical Yield: Application to the Di-π-Methane and Norrish Type II Photorearrangements

Stephen V. Evans, Miguel Garcia-Garibay, Nalamasu Omkaram, John R. Scheffer,* James Trotter,* and Fred Wireko

Department of Chemistry, University of British Columbia Vancouver, Canada V6T 1Y6

Received March 6, 1986

A material that crystallizes in a chiral space group is characterized by the fact that the environment of the molecules is chiral. All optically pure compounds necessarily crystallize in chiral space groups, and a few examples are known of racemic mixtures that behave similarly, the most famous being that studied by Pasteur[1] of racemic sodium ammonium tartrate, which crystallizes below 28 °C as a mixture of enantiomeric crystals that can be differentiated by their morphology and separated by hand. In cases where the enantiomers are in equilibrium under the crystallization conditions, it is sometimes possible to convert the entire sample to crystals composed of a single enantiomer without the influence of an external asymmetric agent. An example of this genuine "spontaneous resolution" was provided by Pincock in his studies on binaphthyl.[2] Examples are also known of achiral molecules

(1) For an excellent discussion of Pasteur's work with sodium ammonium tartrate, see: Fieser, L. F.; Fieser, M. *Advanced Organic Chemistry*; Reinhold: New York, 1961; p 69.
(2) (a) Pincock, R. E.; Wilson, K. R. *J. Am. Chem. Soc.* **1971**, *93*, 1291-1292. (b) Pincock, R. E.; Perkins, R. R.; Ma, A. S.; Wilson, K. R. *Science (Washington, D.C.)* **1971**, *174*, 1018-1020. Other examples of spontaneous crystallization of one of two rapidly interconverting enantiomers from solutions are described by: (c) Havinga, E. *Biochim. Biophys. Acta* **1954**, *13*, 171-174. (d) Newman, A. C. D.; Powell, H. M. *J. Chem. Soc.* **1952**, 3747-3751.

0002-7863/86/1508-5648$01.50/0