Two features of the presently available data suggest that the association and conformational equilibria responsible in part for the enantiomeric shift differences are complex. First, there is no apparent correlation between the magnitudes of the shifts induced in chemically distinct sets of enantiomeric protons and the structure of the shift reagents. Thus, the shift between the methyl protons of (*R*)- and (*S*)-1-phenylethylamine is small in solutions of **2** but moderately large in the presence of **3**, while the C*H* protons in the same samples show the opposite behavior (Figure 1). Second, the *sense* of the shift difference need not be the same for all the protons in enantiomeric substrates; each proton in (*R*)-1-phenylethylamine falls at lower field than the corresponding proton of the *S* enantiomer in solutions containing **1**,[2] while the protons of the methyl group of the *R* enantiomer resonate at *higher* field, and the C*H* of this enantiomer at *lower* field, than the analogous *S* protons, in the presence of **2** and **7**. Since the shielding experienced by protons in complexes of lanthanide shift reagents with substrates is a sensitive function of the geometry of these complexes,[6,7] and since the diastereomeric complexes formed as the result of coordination of enantiomeric bases to chiral chelates need not necessarily have closely related geometries, these observations are not surprising. However, they do suggest that *prediction* of the sense and relative magnitudes of shifts between enantiomers in these systems will be difficult,[8] and that in practical applications it may accordingly be worthwhile to examine several different chiral shift reagents to find empirically the one giving the most useful spectra.[8a]

Acknowledgment. We wish to thank Professor Harlan Goering for communicating results prior to publication[9] and for samples, and Hoffmann-LaRoche, Inc., for gifts of optically active compounds.

---

[6] B. L. Shapiro, J. R. Hlubucek, C. R. Sullivan, and L. F. Johnson, *J. Amer. Chem. Soc.*, **93**, 3281 (1971); P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, **92**, 5734, 5737 (1970), and references in each.

[7] For recent structural data on tris(β-diketo)lanthanide complexes, see J. C. A. Boeynes, *J. Chem. Phys.*, **54**, 75 (1971); W. D. Horrocks, Jr., J. P. Sipe III, and J. R. Luber, *J. Amer. Chem. Soc.*, **93**, 5258 (1971), and references in each.

[8] The prospects for utilizing chiral shift reagents to determine *absolute* configurations presently appear very dim. For successful applications of nmr spectroscopy in chiral solvents to determination of absolute configurations, see W. H. Pirkle, R. L. Muntz, and I. C. Paul, *ibid.*, **93**, 2817 (1971).

[8a] NOTE ADDED IN PROOF. Additional evidence supporting the hypothesis that the conformation of the β-diketone ligands around the europium ion is fairly flexible is provided by the observation by M. McCreary that it is possible to *induce* chirality in Eu(DPM)$_3$ by coordination with a chiral amine. Thus, a $\Delta\Delta\delta$ of 0.36 ppm is observed for the C*H*NH$_2$ resonance of racemic 1-phenylethylamine (0.45 *M*) in a CDCl$_3$ solution containing Eu(DPM)$_3$ (0.45 *M*) and (*R*)-*N*-methyl-1-phenylethylamine (0.64 *M*).

[9] H. L. Goering, J. N. Eikenberry, and G. S. Koermer, *ibid.*, **93**, 5913 (1971).

George M. Whitesides,* Daniel W. Lewis
Department of Chemistry, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received August 3, 1971

Experimental Evidence for the Assignment of α C*H* Peaks in the Nuclear Magnetic Resonance Spectra of Polypeptides

Sir:

In pmr spectra of polypeptides undergoing the helix–coil transition, the backbone resonances α C*H* and N*H* sometimes give rise to double rather than single peaks. Although these are usually assigned to largely coil and largely α helical conformations, two recent publications, one from Scheraga's laboratory[1] and the other from Tam and Klotz,[2] have proposed different assignments. Evidence is provided here to support the original assignment of the two peaks, first observed by Ferretti.[3]

If it is assumed that the helical and random coil conformations are both present in single polypeptide chains, the observation of double α C*H* peaks suggests that the helix–coil transition is a slow exchange phenomenon ($\tau \approx 10^{-2}$ sec).[4] This is in sharp contrast to the results of several relaxation experiments[5] on the transition that indicate fast exchange ($\tau \approx 10^{-7}$–10^{-8} sec).[5] Explanations of the double peak behavior based on slow exchange have been given by Bradbury and coworkers,[6] who propose protonation of the amide residues, and by Ferretti and coworkers,[7] who propose a nucleation step having a high potential energy barrier. Ullman[8] has shown, however, that it is not necessary to postulate slow exchange since polydispersity and end-of-chain effects are sufficient to give rise to multiple peaks even with rapid exchange. Experimental evidence has been presented by ourselves which supports the ideas of Ullman.[9] Recently, however, Scheraga and coworkers[1] and Tam and Klotz[2] have questioned the assignment of the double peaks. The former authors[1] assume that the resonance of α helical backbone protons will not be observable due to dipolar broadening and propose that the two peaks observed are due to acid-solvated and unsolvated coil residues. The latter authors,[2] on the basis of polyalanine spectra, reintroduce the suggestion of protonation by TFA and favor the proposals of Bradbury and coworkers[6] as to the origin of the double peak.

Scheraga's proposals may be simply tested as follows: if the resonance from helical residues is too broad to be observed then the α C*H* peak area observed in helix-supporting solvents represents only a small fraction of the total polymer and corresponds to the few residues remaining in an "unsolvated coil" state. Addition of strong acid causing a complete helix–coil transition should therefore result in a large increase in relative area of the peak at the chemical shift attributed to "solvated coil," since all residues will then contribute to the peak area. The same change in solvent composition should cause a much smaller effect on the areas of the side-chain peaks and Table I, therefore, gives the ratios of the total α C*H* area to that of

---

[1] F. J. Joubert, N. Lotan, and H. A. Scheraga, *Biochemistry*, **9**, 2197 (1970).

[2] J. W. O. Tam and I. M. Klotz, *J. Amer. Chem. Soc.*, **93**, 1313 (1971).

[3] J. A. Ferretti, *Chem. Commun.*, 1030 (1967).

[4] (a) J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967); (b) E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Nature (London)*, **216**, 862 (1967); (c) J. A. Ferretti and L. Paolillo, *Biopolymers*, **7**, 155 (1969); (d) J. H. Bradbury and M. D. Fenn, *Aust. J. Chem.*, **22**, 357 (1969).

[5] (a) R. Lumry, R. Legare, and W. G. Miller, *Biopolymers*, **2**, 484 (1964); (b) T. K. Saksena, B. Michels, and R. Zana, *J. Chim. Phys. Physicochim. Biol.*, **65**, 597 (1968); (c) G. Schwarz and J. Seelig, *Biopolymers*, **6**, 1263 (1968).

[6] J. H. Bradbury, M. D. Fenn, and A. G. Moritz, *Aust. J. Chem.*, **22**, 2443 (1969).

[7] J. A. Ferretti, B. W. Ninham, and V. A. Parsegian, *Macromolecules*, **3**, 34 (1970).

[8] R. Ullman, *Biopolymers*, **9**, 471 (1970).

[9] E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, **11**, 277 (1970).

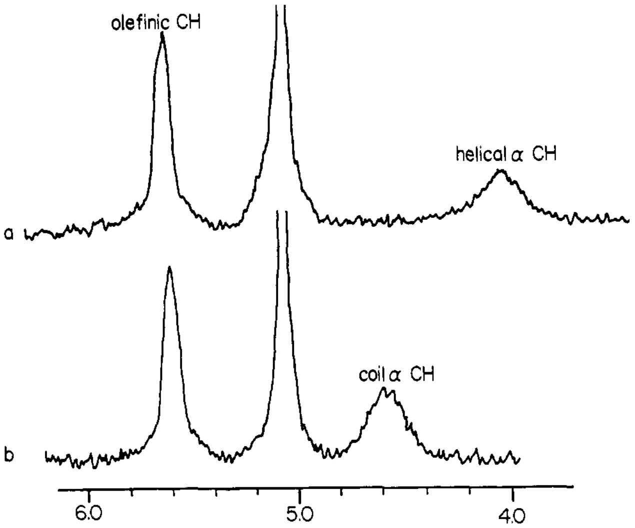


Figure 1. Spectra (100-MHz) of poly-γ-benzyl-L-glutamate comparing the α C*H* of helix and random coil forms with an olefinic C*H* internal intensity reference: (a) helix in $CDCl_3$–5% TFA; (b) random coil in $CDCl_3$–15% TFA.

two side-chain peaks. The poly-γ-benzyl-L-glutamate (PBLG) sample was of average DP (~100) and gave a well-defined double peak at the mid-transition point of 7% TFA. With the exception of neat chloroform (in which the polypeptide aggregates) the peak ratios are very constant and close to the expected values. In addition a direct comparison has been made of the total α C*H* area through the transition with respect to an internal nonpolymeric reference compound. This gives an absolute evaluation of the number of protons observed in the α C*H* polymer spectrum. 1,5-Cyclooctadiene was chosen since the olefinic protons give a broad peak in a region free of polymer peaks. In spectrum b of Figure 1 (random coil from ORD) the α C*H* area corresponded to 84% of the calculated, while in the helical spectrum a the measured area represented 77%. These are lower limits due to the difficulty of estimating the areas of the long wings of broad peaks. Within the limits of the experiment the area of the helical α C*H* peak is thus close to that of the random coil and the interpretation of Joubert, *et al.*,[1] is therefore untenable.

Table I. Area Ratios of α C*H* Peak to Side-Chain Peaks of a Sample of PBLG[a]

v/v % TFA	α-CH/benzyl-CH_2	α-CH/(β + γ)-CH_2
0	0.26	0.16
1	0.47	0.24
7	0.47	0.23
15	0.47	0.23

[a] Mol wt = 22,100; in $CDCl_3$–TFA mixtures, as measured by the spectrometer integrator (Varian Associates HA-100-15D). Each measurement is the average of at least four integrations.

The recent letter by Tam and Klotz[2] suggests that since low molecular weight poly-L-alanine and poly-DL-alanine in $CDCl_3$–TFA both appear to show double α C*H* peaks, the usual assignment to helix and coil cannot be correct since the racemic polymer could not have a significant helix content. They moreover consider their spectra as supporting the proposed protonation of amide groups. Without wishing to discuss the possible conformations of DL-polypeptides we wish to point out that poly-L- and -DL-alanine normally[10–12] show a single α C*H* peak which shifts downfield on increasing the acid content (TFA) from ~30%, the minimum required for solubility in chloroform. The samples of polyalanine studied by Tam and Klotz[2] were unique in that they dissolved in pure chloroform and we have found it necessary to synthesize block copolymers of L-alanine flanked by solvating polypeptide in order to study the low-TFA region of the transition.[13] The spectrum of Figure 2 shows that the α C*H* of the L-alanine block is a single peak at 4.16 ppm (corresponding to the low-field peak of Tam and Klotz) and that there is no sign of their high-field peak at 3.87 ppm. Only one peak is observed throughout the transition to the coil. In none of our poly-L-alanine samples, prepared either as a homopolymer or as a block copolymer, has double peak behavior been observed. In view of these experimental differences, which stem presumably from differences in the material being studied, it seems premature to base any general conclusions concerning the nmr manifestation of the helix–coil transition on spectra of polyalanine.

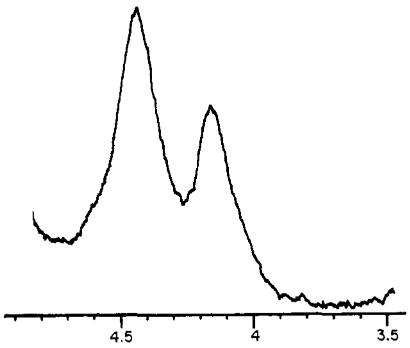


Figure 2. A 220-MHz spectrum of the α C*H* region of block copoly[(benzyl-L-aspartate)$_{40}$(L-alanine)$_{44}$(benzyl-L-aspartate)$_{36}$] in $CDCl_3$–0.9% TFA; aspartate α C*H* peak at 4.44 ppm and alanine α C*H* peak at 4.16 ppm.

A successful theory of this spectral phenomenon must explain why (1) high molecular weight PBLG exhibits a single-shifting α C*H* peak which broadens at high helicity, while low molecular weight PBLG shows a double peak spectrum with the more polydisperse samples showing a more pronounced peak separation; (2) fractionation of the polymer considerably reduces double peak character; (3) double peak character has also been observed in D_2O–methanol (poly-L-arginine[14]) and D_2O–DMSO (poly-L-tyrosine).[15] Only the proposals of Ullman are sufficiently wide in their application to account for all the observations so far made.

---

[10] W. E. Stewart, L. Mandelkern, and R. E. Glick, *Biochemistry*, **6**, 143 (1967).

[11] E. M. Bradbury and H. W. E. Rattle, *Polymer*, **9**, 201 (1968).

[12] J. A. Ferretti and L. Paolillo, *Biopolymers*, **7**, 155 (1969).

[13] E. M. Bradbury, P. Cary, and C. Crane-Robinson, manuscript in preparation.

[14] M. Boublik, E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Eur. J. Biochem.*, **12**, 258 (1970).

[15] E. M. Bradbury, C. Crane-Robinson, V. Giancotti, and R. M. Stephens, *Polymer*, in press.

Acknowledgments. Support from the S.R.C. of Great Britain and the C.N.R. of Italy is gratefully acknowledged by the authors.

E. M. Bradbury,* P. Cary, C. Crane-Robinson
Biophysics Laboratories, Physics Department
Portsmouth Polytechnic
Portsmouth P01 2DZ, England

L. Paolillo, T. Tancredi, P. A. Temussi
Istituto Chimico, University of Naples, Naples, Italy
Laboratorio per la Chimica e Fisica di
Molecole di Interesse Biologico del C.N.R.
Arco Felice, Naples, Italy

Received April 26, 1971

Photochemistry of Cyclopropanone

Sir:

The nature of primary photoprocesses important in the larger cyclic ketones has provoked considerable discussion in recent years, with the formation of biradical intermediates,[1] intersystem crossing to a triplet state,[2,3] internal conversion to the ground electronic state,[4] and predissociation[5] all having been invoked to explain diverse results of different workers. We are in the process of a thorough study of the photochemical and photophysical pathways in cyclopropanone which we hope will contribute toward the development of a systematic description of the fundamental factors determining the fates of electronically excited cyclic ketones in general. We wish to present here a preliminary report of our photochemical results for cyclopropanone—the first such report for this compound in the gaseous state.[6]

Upon irradiation of samples of gaseous cyclopropanone, at pressures from ~300 μ to 4 Torr and wavelengths from 2920 to 3650 Å, we find ethylene and carbon monoxide to be the only volatile photochemical products. Mass spectrometric analysis of a sample photolyzed to 93% of completion at 3130 Å confirmed that these two products are formed in equal amounts within experimental error (~3%). Other possible products such as acrolein, ketene, and cyclopropanone dimers have been searched for using gas chromatography, ir and uv spectrophotometry, and mass spectrometry without success. We estimate that these compounds could have been detected if they had amounted to as little as 2, 4, and 6%, respectively, of the total volatile products formed in a typical photolysis carried to 10% completion. The quantum yield for disappearance of cyclopropanone, determined by following the decay of absorption in its second uv band at 2057 Å (ϵ 763 M^{-1} cm^{-1}) and using gaseous azomethane as the actinometer,[7] was found to be 1.0 within experimental error at all pressures and wavelengths. In contrast, the quantum yield of ethylene formation is wavelength dependent, varying from 0.59 at 3650 Å to 0.98 at 2920 Å. Addition of up to 1270 Torr of CO_2, 420 Torr of O_2, and 650 Torr of 1,3-butadiene had no effect upon the quantum yields for ethylene or for cyclopropanone (except that ϕ_{cp} could not be measured in the presence of butadiene) at any wavelength of irradiation. In the case of added butadiene, the adduct between a cyclopropanone biradical and butadiene was looked for by means of gas chromatography but not found.

The averaged experimental conditions and results of ten photolyses carried out with no added gas are summarized in Table I. The 3650- and 3130-Å lines from a high-pressure mercury arc were isolated by interference filters and the 3341- and 2920-Å lines by a Bausch and Lomb high-intensity monochromator. The amount of ethylene formed was determined by gas chromatography with corrections for ~0.5% C_2H_4 impurity based on analysis of the photolysis blanks. The cyclopropanone used was prepared by a flow system modification of the method of Turro and Hammond,[8] purified by repeated trap-to-trap distillation on a high-vacuum line, and had ≤1% cyclobutanone impurity in the samples used for the data reported here.

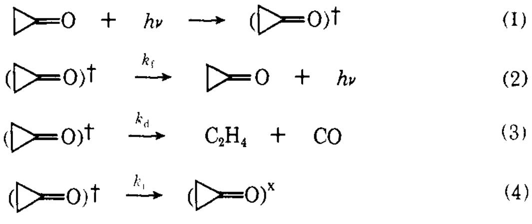
Table I. Cyclopropanone Photolysis Data[a]

λ, Å	$P_{0,cp}$, μ	I_0, photons sec^{-1}	Photolysis time, min	$\Delta P_{C_2H_4}$, μ	$\phi_{C_2H_4}$	ϕ_{cp}
3650[b]	790	7.32×10^{15}	62	41.7	0.59 ± 0.02	0.90 ± 0.07
3341	762	1.41×10^{15}	60	35.2	0.64	0.97
3130[c]	723	1.30×10^{16}	14	92.7	0.72 ± 0.01	1.02 ± 0.05
2920[d]	719	7.08×10^{14}	90	28.4	0.98 ± 0.06	0.99 ± 0.07

[a] Temperature 23°, photolysis cell volume 29.7 μ. Error ranges stated are mean deviations. Estimated limits of error for individual values are somewhat larger (*e.g.*, ±0.04 for $\phi_{C_2H_4}$ and ±0.17 for ϕ_{cp} at 3650 Å). [b] Average of three photolyses. [c] Average of four photolyses, including ones at 348 and 4340 μ (excluded from P_0 average). [d] Average of two photolyses.

Figure 1 shows the near-uv absorption spectrum of gaseous cyclopropanone, with arrows indicating the wavelengths of irradiation used. Some diffuse vibrational structure is indicated, ending near the shortest λ_{irr} of 2920 Å. Using the recent analysis of the spectrum of cyclobutanone[9] as a guide, we estimate the 0–0 band in this transition to lie near 3950 Å. Thus the vibrational energy of the electronically excited cyclopropanone produced by the different wavelengths of irradiation would range from ~6.0 kcal mol^{-1} at 3650 Å to ~25.5 kcal mol^{-1} at 2920 Å.

A simple mechanism which accounts for the observed products and quantum yields in the photolysis of gaseous cyclopropanone is



---

(1) R. L. Alumbaugh, G. O. Pritchard, and B. Rickborn, *J. Phys. Chem.*, **69**, 3225 (1965).

(2) E. K. C. Lee, *ibid.*, **71**, 2804 (1967).

(3) J. C. Dalton and N. J. Turro, *Annu. Rev. Phys. Chem.*, **21**, 499 (1970).

(4) N. E. Lee and E. K. C. Lee, *J. Chem. Phys.*, **50**, 2094 (1969).

(5) J. C. Hemminger and E. K. C. Lee, *ibid.*, **54**, 1405 (1971).

(6) Unpublished work by N. J. Turro and W. B. Hammond showed C_2H_4 and CO to be produced upon irradiation of a solution of cyclopropanone in CH_2Cl_2 at −78°; see W. B. Hammond, Ph.D. Thesis, Columbia University, 1967, p 111.

(7) C. V. Cannon and O. K. Rice, *J. Amer. Chem. Soc.*, **63**, 2900 (1941); S. Toby and B. H. Weiss, *J. Phys. Chem.*, **68**, 2492 (1964).

(8) N. J. Turro and W. B. Hammond, *J. Amer. Chem. Soc.*, **88**, 3672 (1966).

(9) D. C. Moule, *Can. J. Phys.*, **47**, 1236 (1969).