The Chemiluminescence of Grignard Reagents. Some Spectroscopic and Mechanistic Studies

Richard L. Bardsley and David M. Hercules

Contribution from the Department of Chemistry and Laboratory for Nuclear Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

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Abstract: Chemiluminescence spectra have been obtained by oxidation of dilute Grignard solutions in benzene–triethylamine solvent. The spectra differ considerably from those of earlier workers who obtained spectra from more concentrated solutions. The emitting species in the chemiluminescent oxidation of phenylmagnesium bromide has been identified as *p*-terphenyl; three emitters were observed for *p*-chlorophenylmagnesium bromide. The results of the present investigation are used to explain some structural and energy correlations of earlier workers. Possible mechanisms are discussed for the chemical excitation of *p*-terphenyl.

The chemiluminescent reaction between Grignard reagents and oxygen has received little attention during the last 30 years. Earlier investigations were largely concerned with qualitative or semiquantitative aspects of the phenomenon.[1–7] Only one modern physicochemical study of Grignard chemiluminescence has been reported.[8] In addition, the effects of high-voltage electrolysis (*ca.* 1 kV) and magnetic fields (15 kg) on the chemiluminescence of Grignard compounds have been studied.[9] Some aspects of earlier work on the chemiluminescence of Grignard compounds have been reviewed by Kharash and Reinmuth in their monumental treatise[10] and by two recent reviews on chemiluminescence.[11,12]

Several things about the earlier investigations prompted us to study the chemiluminescence of Grignard oxidation. First, the emitting species had not been identified for any reaction, and such identification is a necessity for mechanistic studies. Second, the reported spectra showed major changes with small variations in the organic moiety which did not seem to be characteristic of well-known correlations between aromatic substitution and spectra. Third, it was difficult to understand how chemiluminescent reactions of simple phenyl derivations could cause virtually all of their light to be emitted in the visible region of the spectrum.

The present communication reports a study of the chemiluminescence spectra of phenylmagnesium bromide and *p*-chlorophenylmagnesium bromide. We have been able to identify *p*-terphenyl as the major emitting species in the chemiluminescent oxidation of phenylmagnesium bromide. We are able to report some observations relating to the chemical excitation of *p*-terphenyl; these allow us to propose two possible mechanisms for excitation and to reject several others. We are also able to explain some of the generalizations from earlier work[10] although these latter phases of the investigation are far from complete.

Experimental Section

Grignard Reagents. Phenylmagnesium bromide and *p*-chlorophenylmagnesium bromide, approximately 1 *M*, were prepared by conventional procedures under nitrogen atmospheres using deoxygenated solvents. Benzene, containing 1 mole of triethylamine per mole Grignard,[13] was the preferred solvent. *n*-Butyl ether preparations gave identical chemiluminescence spectra but more complex oxidation products and were more difficult to dilute.

Analysis of Grignards. The solutions prepared were analyzed for active Grignard by the acid titration method of Gilman,[14] and yields in the range 70–80% were indicated. Samples hydrolyzed before or after oxidation, or after reaction with 2,6-dimethyl-4-heptanone, were analyzed for biphenyl and *p*-terphenyl by gas–liquid chromatography of the evaporated organic layers on a 10% DC 11 silicone oil column on 60–80 mesh Chromosorb W using an Aerograph 1620 gas chromatograph.

Oxidation Product Separations. The Grignard reagents were shaken under an atmosphere of dry oxygen for several hours and hydrolyzed, and the separated organic layers were dried over magnesium sulfate.

1. Phenylmagnesium Bromide. Samples (1 ml) were fractionated in the Aerograph Autoprep 700 gas chromatograph using a 20-ft column of 30% SE 30 silicone rubber on Chromosorb W at 250°. Fluorescence of the collected products was monitored using both long-wavelength and short-wavelength hand lamps.

Thin-layer chromatograms using standard procedures for *n*-heptane elution on Eastman 6060 silica gel sheets showed one fluorescent spot from the oxidation residues.

2. *p*-Chlorophenylmagnesium Bromide. No strongly fluorescent compounds could be isolated from the reaction product by gas chromatography at temperatures up to 300°. Thin-layer chromatography using heptane, chloroform, and *n*-heptane–chloroform mixtures as eluents showed a series of unresolvable, blue fluorescent spots. Adsorption chromatography using an alumina column and gradient elution with *n*-heptane–chloroform mixtures separated three blue fluorescent bands.

Fluorescence and Absorption Spectra. Fluorescence spectra of product solutions and separated products were obtained using a G. K. Turner Model 210 absolute spectrofluorometer.[15] Absorption spectra were obtained on the Cary 14 spectrophotometer.

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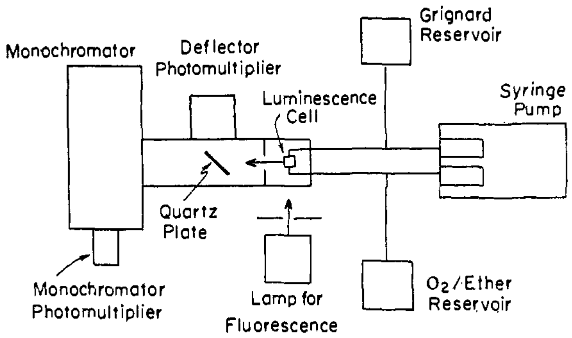

Figure 1. Block diagram of spectrometer used for obtaining chemiluminescence spectra.

Dilution of the Grignards. Benzophenone ketyl was generated in benzene solution by the addition of triethylamine, sodium metal, and benzophenone. This oxygen-free benzene was distilled directly into a reservoir (containing the Grignard solution) surrounded by a nitrogen-filled glove bag. The concentrated Grignard was transferred to the reservoir by pipet inside the glove bag.

Chemiluminescence Spectra. Chemiluminescence was produced by pumping solutions of the Grignard and oxygen-saturated butyl ether through a glass T-shaped mixer–luminescence cell. Syringes were filled *via* three-way taps from separatory funnels used as solution reservoirs maintained under nitrogen and oxygen atmospheres, respectively.

Chemiluminescence Spectrometer. A diagram of the chemiluminescence spectrometer is shown in Figure 1. The light path between the luminescence cell and the Bausch & Lomb grating monochromator was interrupted by a thin, transparent, quartz plate held at 45° to the incident light in order to deflect a fixed fraction at right angles. An RCA 1P21 photomultiplier placed in the deflected beam monitored the intensity of the light emitted from the chemiluminescence cell. Light emerging from the monochromator exit slit was measured by a similar photomultiplier.

Photomultiplier anode pulses were amplified by a cathode-follower preamplifier circuit and counted by EKCO NS30F and Baird Atomic 132 automatic scalars. The rate of counting observed is proportional to the rate of photon impingement on the photomultiplier.[16]

With the monochromator set at a wavelength, λ, the chemiluminescent reaction was run for 100 sec with both photomultiplier counter readings. After subtraction of the dark counts, the ratio of the two readings, monochromator PM counts/deflector PM counts, was recorded.

This procedure, repeated for different wavelengths without altering the position of the luminescence cell, enabled a complete spectrum to be obtained. Such an integration method is advantageous for luminescence of low or fluctuating intensity, but the points recorded are subject to statistical errors resulting from the random spacing of the photomultiplier output pulses.

Fluorescence spectra were also taken by illuminating a quartz cell at 90° to the path of the emission detection system. Fluorescent and chemiluminescent spectra are not corrected for monochromator transmission efficiency or photomultiplier spectral response.

Comparison of Luminescence Intensities. The intensity of the chemiluminescence produced at the center of the mixer used in the previous experiments was monitored by placing the mixer in front of a 1P21 photomultiplier and separated from it by a slit. The *p*-terphenyl concentration in the Grignard solution was increased by adding measured amounts of an oxygen-free benzene solution of *p*-terphenyl.

The photomultiplier output was calculated from the voltage drop across a variable load resistor as measured by a 10-mV potentiometric recorder.

Results

The oxidation products of phenylmagnesium bromide were separated by preparative-scale gas chromatography. Fluorescence spectra of the isolated fractions were obtained, and one of these matched the fluorescence spectrum of the Grignard solution after oxidation. The material contained in the fraction was identified as *p*-terphenyl by comparison of infrared, ultraviolet, and mass spectra with those of an authentic sample. Thin layer chromatography also confirmed that *p*-terphenyl alone was responsible for the strong fluorescence of the product solution.

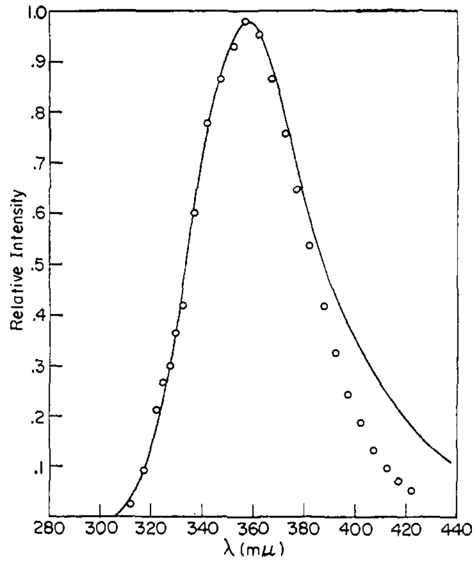

Figure 2. Emission spectra for phenylmagnesium bromide in benzene–triethylamine: ——, chemiluminescence spectrum for reaction with oxygen-saturated *n*-butyl ether; ∘ ∘ ∘, fluorescence spectrum of *p*-terphenyl and of Grignard solution after oxidation.

Gas chromatographic analysis of a 0.79 *M* phenylmagnesium bromide solution in benzene–triethylamine showed the presence of 6.2×10^{-2} *M* biphenyl and 3.4×10^{-4} *M* *p*-terphenyl before oxidation. After oxidation the biphenyl concentration had not changed significantly, while the *p*-terphenyl concentration was found to be 6.8×10^{-4} *M*. The original concentrations of biphenyl and *p*-terphenyl were unaltered on reaction of the Grignard with 2,6-dimethyl-4-heptanone, indicating the absence of large amounts of the metalated Grignards, biphenylmagnesium bromide and *p*-terphenylmagnesium bromide, reported in the preparation of nonsolvated phenylmagnesium bromide.[17] When a Grignard solution was prepared using 0.5 mole of *p*-terphenyl and 1.0 mole of bromobenzene per liter, no large quantities of the *p*-terphenyl Grignard could be detected.

The chemiluminescence spectrum shown in Figure 2 was obtained for the reaction of oxygen-saturated *n*-butyl ether with 2.2×10^{-3} *M* phenylmagnesium bromide in benzene–triethylamine solvent. This was the lowest concentration at which spectra were obtainable with the equipment used. This spectrum is corrected for solution absorption but not for photomultiplier response or monochromator transmission efficiency. Figure 2 also shows a comparison between the fluorescence spectrum of *p*-terphenyl (10^{-6} *M* in *n*-butyl ether) and the chemiluminescence spectrum for the oxidation of phenylmagnesium bromide, both taken on the same spectrometer ($\lambda_{\text{excitation}}$ 300 mμ).

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(16) G. P. Burn, *Electron. Eng.*, 33, 806 (1961).

(17) R. H. F. Manske and A. E. Ledingham, *Can. J. Chem.*, 27, 158 (1949).

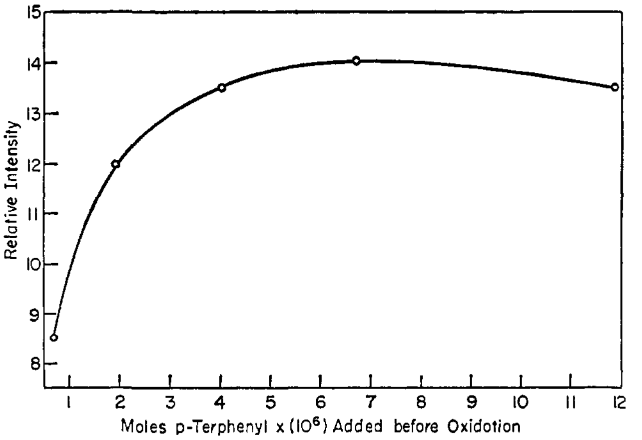


Figure 3. Effect of *p*-terphenyl on the chemiluminescence intensity of phenylmagnesium bromide; *p*-terphenyl added to Grignard solution before oxidation.

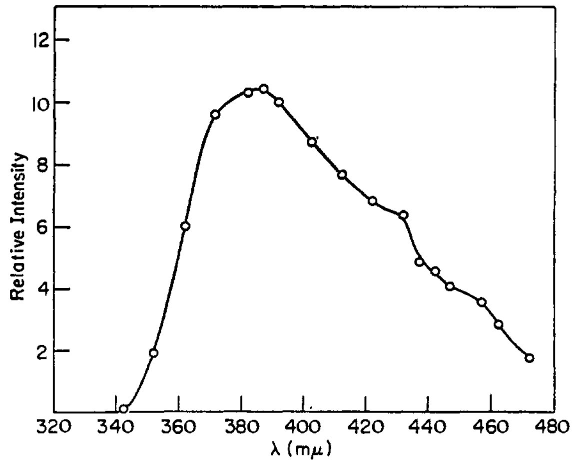
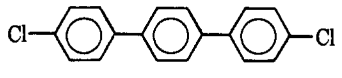


Figure 4. Chemiluminescence spectrum of *p*-chlorophenylmagnesium bromide in benzene–triethylamine; oxygen-saturated *n*-butyl ether used as oxidant.

A chemiluminescence spectrum was obtained for a 2.5×10^{-3} *M* Grignard solution with 2.5×10^{-4} *M* 9,10-diphenylanthracene added to the oxygen-saturated butyl ether solution. Analysis revealed the Grignard solutions contained 1.5×10^{-5} *M* biphenyl and 2.5×10^{-6} *M* *p*-terphenyl, corrected for dilutions in the mixes. The chemiluminescence spectrum showed two peaks, one corresponding to the fluorescence of *p*-terphenyl and the other to the fluorescence of 9,10-diphenylanthracene. Fluorescence spectra obtained on the solutions after oxidation showed the same two peaks when excited at 2850 Å.

The addition of *p*-terphenyl in the range 3×10^{-6} to 5×10^{-5} *M* to the oxygen-saturated butyl ether solution produced no increase in the chemiluminescence intensity of a 10^{-3} *M* Grignard solution. If, however, the *p*-terphenyl was added directly to the Grignard solution *before* oxidation, the intensity of luminescence increased, as shown in Figure 3.

Three distinguishable blue fluorescent fractions were isolated by adsorption chromatography from the oxidation products of *p*-chlorophenylmagnesium bromide. Only one product could be identified and was shown to be a chlorinated terphenyl, probably



Its fluorescence spectrum, similar to that of *p*-terphenyl with a fluorescence maximum at 345 mμ ($\lambda_{\text{excitation}}$ 300 mμ), does not match the chemiluminescence spectrum obtained for a 4×10^{-4} *M* solution of the Grignard in benzene–triethylamine as indicated by Figure 4.

The other two fluorescent products could not be obtained in sufficient quantity to permit identification. They have fluorescence maxima at 377 and 387 mμ, respectively, which correspond closely to the maxima of the chemiluminescence spectra of *p*-chlorophenylmagnesium bromide.

The chemiluminescence spectrum of a commercial sample of phenyllithium[18] at approximately 10^{-3} *M* in benzene was obtained under conditions identical with those of the Grignards. It appeared as a broad band extending from 320 to 600 mμ, with peaks at 350 and 530 mμ. Even at this concentration, the solution was a deep brown color, causing considerable distortion of the spectrum by self-absorption and making the spectrum of questionable value.

Discussion

Spectra of Grignards. Figure 2 indicates that there is a good match between the chemiluminescence spectrum of phenylmagnesium bromide (I), the fluorescence spectrum of a solution of I after oxidation, and the fluorescence spectrum of *p*-terphenyl (II). The spectral match, coupled with the observation that II is the only major fluorescent product isolated after oxidation of I, constitutes strong evidence that *p*-terphenyl is the emitter in the chemiluminescent oxidation of phenylmagnesium bromide.

It should be noted that there is a mismatch on the long-wavelength side of the spectra shown in Figure 2. This can be attributed to the presence of small amounts of emitters other than II which could not be isolated by our techniques but which could be excited during the chemiluminescent oxidation. For example, hydroxylated polyphenyls have been observed in the oxidation of I in ether.[19]

The results reported here differ from those of earlier workers who observed that chemiluminescence emission occurred mostly in the visible region of the spectrum[3,4] and that chemiluminescence spectra did not match the fluorescence spectra of solutions after oxidation.[1,2,4] The reason for these discrepancies is that earlier workers obtained their spectra from Grignard solutions of *ca.* 1 *M* concentration while our solutions were *ca.* 10^{-3} *M*. In the concentrated Grignards self-absorption of the short-wavelength components of the spectra would occur, badly distorting the spectrum. In fact, even in the dilute solutions used here it was necessary to apply a small self-absorption correction. As we increased the concentration of our Grignard reagents, the spectra became distorted and the maxima showed red shifts, resulting in spectra which more nearly matched those of earlier workers. The discrepancies between fluorescence and chemiluminescence spectra can also be explained by self-absorption; in the apparatus used

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by earlier workers the fluorescence and chemiluminescence were viewed through different depths of solution.

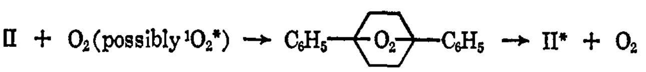
Since the 0,0 band of *p*-terphenyl probably lies at about 310 mμ, the energy required for the chemical excitation of II is very high, *ca.* 92 kcal/mole. To the best of our knowledge this makes the oxidation of phenylmagnesium bromide the most energetic solution chemiluminescent reaction yet reported, having a major portion of its emission lying in the ultraviolet.

Thomas and Dufford have measured efficiencies for the chemiluminescence of I and III.[7] They defined efficiency as the ratio of the total luminous flux of light from the chemiluminescent reaction to the heat of reaction. Their values were 1.3×10^{-9} for I and 2.3×10^{-7} for III. Considering that their measurements were obtained on concentrated solutions and that they measured what is best regarded as an energy efficiency, the quantum efficiencies (*i.e.*, probability of excited state formation during oxidation) would probably be *ca.* 10^3 larger or 10^{-6} and 10^{-4} for I and III, respectively. These values agree with our qualitative observations of efficiency, relative to the luminol reaction.

Possible Chemiluminescent Mechanisms. There are two types of mechanisms one might consider for the chemiluminescence of phenylmagnesium bromide: first, where II serves as an energy acceptor; and, second, where II is produced in an excited state by a chemical reaction occurring during the oxidation process.

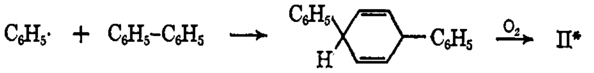
A mechanism of the first type might involve energy transfer to II from either a singlet oxygen dimer[20] or a benzene triplet. Either of these species could be produced by decomposition of benzene hydroperoxide, C_6H_5OOH, which would arise from solvent reaction with $C_6H_5OOMgBr$.[21-23] Neither of these mechanisms is likely, from energy considerations, since the singlet oxygen dimer has available only *ca.* 83 kcal/mole (including two vibrational quanta)[20] and triplet benzene only 84 kcal/mole,[24] 92 kcal/mole being required to excite the lowest singlet of II. Although it is possible that energy transfer to the triplet of II could occur, two other observations rule out such a mechanism. First, addition of a 100-fold excess of 9,10-diphenylanthracene did not significantly quench the emission observed for II, as would be expected if II were serving simply as an acceptor in an energy-transfer process. Second, addition of II to the oxygen–ether solutions did not increase the intensity of emission from II during the chemiluminescent oxidation. Addition to the ether solution ensured that the added II could not become involved in reactions within the Grignard solution prior to oxidation.

A mechanism of the second type might be decomposition of *p*-terphenyl peroxide produced by reaction of II during the oxidation.



Such a mechanism also can be ruled out because the addition of II to the oxygen–ether solution did not increase the intensity of chemiluminescence. It is also likely that addition of 9,10-diphenylanthracene would have quenched emission of II if the peroxide mechanism were operative, due to competitive peroxide formation.

Another possible mechanism might involve a radical coupling reaction and subsequent oxidation, such as



Such a reaction would involve biphenyl as a precurser, and, because biphenyl added to the oxygen–ether solution failed to increase chemiluminescence intensity, such a mechanism seems unlikely.

Figure 3 indicates that chemiluminescence intensity is a function of the concentration of II if it is added to the Grignard solution prior to oxidation, but the intensity is not a function of II if it is added to the oxygen–butyl ether solution. This implies there is a reaction involving II in the Grignard solution before oxidation which produces some species capable of giving excited II on oxidation. There are two types of reactions which could account for these results, metalation and ionization.

Although extensive metalation between I and II has not been reported, several studies indicate such a reaction to be likely. Gilman and Bebb observed that metalation between biphenyl and butyllithium is extensive, up to 15% of the biphenyl in solution.[25] McCoy and Allred found exchange between methyl groups in compounds of the type $(CH_3)_2M$ with exchange lifetimes of *ca.* 0.2 sec.[26] Challenger and Miller have found evidence for metalation in Grignard–aromatic systems,[27] and Mulvaney, *et al.*, have found similar results for organolithium compounds.[28] Dessy, *et al.*,[29] have indicated it should occur in the case of Grignards. Bryce-Smith and Wakefield report that Grignard reagents can be used for direct alkylations and arylations of aromatic hydrocarbons.[30]

Metalation reactions occurring in the Grignard solution between I and II could easily account for formation of the *p*-terphenyl Grignard

$$C_6H_5MgBr + II \longrightarrow C_6H_6 + C_6H_5\text{—}C_6H_4\text{—}C_6H_4MgBr$$

Oxidation of the *p*-terphenyl Grignard and subsequent reaction with the solvent could produce the *p*-terphenyl hydroperoxide. Chemiluminescence from peroxide decomposition reactions is known, and peroxides are postulated as intermediates in a number of chemiluminescent systems.[11,12]

One difficulty with the metalation mechanism is the inability to detect metalation in our solutions even when large amounts of II were added to the solution during Grignard formation. However, the analytical techniques employed would not be sensitive to a small amount of metalated product. This, coupled with the low chemiluminescent yield of Grignard oxidation,

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does not permit us to exclude oxidation of *p*-terphenyl Grignard as a possible chemiluminescent mechanism.

Formation of hydrocarbon anions in Grignard solutions is not well documented, although there is some evidence that it does occur. Brown and Jones observed that butyllithium reacts with hydrocarbons to give anions in good yield.[31] Winkler and Winkler[32] also have studied electron transfer between phenyllithium and aromatic hydrocarbons. Russell, *et al.*,[33] have studied electron-transfer reactions between a variety of donors and acceptors including *n*-butylmagnesium bromide and butyllithium as donors. Acceptors were aromatic systems such as benzofuran, phenazine, benzo[*c*]cinnoline, and acridine. The extent of transfer varied from *ca.* 1% or less to 60% ion formation, as did the time to reach equilibrium, although in all cases it appeared the reaction was slow, *ca.* 5 min or longer.

Anion formation in solutions of I could occur as follows.

$$C_6H_5MgBr + II \longrightarrow C_6H_5\cdot + [C_6H_5{-}C_6H_4{-}C_6H_5]^-MgBr^+$$

The *p*-terphenyl anion thus formed could react with oxygen or peroxide to give chemiluminescence. Chemiluminescent reactions between peroxides and radical anions of aromatic hydrocarbons are well known.[34]

The hydrocarbon anion oxidation mechanism is consistent with all observations of the present study. Only small amounts of anions would need to be formed since the chemiluminescence efficiency is low. The long exchange lifetimes reported for Grignards and aromatic compounds[33] are consistent with the observed effect of II on the chemiluminescent intensity.

Acknowledgment. This work was supported in part through funds provided by the U. S. Atomic Energy Commission under Contract AT(30-1)-905.

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The Stability of the Polyglutamic Acid α Helix[1,2]

David S. Olander and Alfred Holtzer

Contribution from the Department of Chemistry, Washington University, St. Louis, Missouri. *Received February 12, 1968*

Abstract: The intrinsic mixed acidity constant (pK_0) of glutamic acid polymers has been determined from potentiometric titrations of poly-DL-glutamic acid in aqueous solutions of various NaCl concentrations and at two temperatures (25 and 48°), thus making the difficult extrapolations of poly-L-glutamic acid titration curves more certain. These experiments indicate that pK_0 is nearly independent of temperature but varies from 4.58 in 0.01 *M* NaCl to 4.32 in 0.40 *M* NaCl. Similar studies of the titration of poly-L-glutamic acid as a function of temperature and NaCl concentration give the standard Gibbs free-energy change per amino acid residue for the transition from un-ionized α helix to un-ionized random coil ($\Delta G°/N$) for each of the various conditions; $\Delta G°/N$ depends on temperature, but only slightly, if at all, on salt concentration. From the temperature dependence of the free energy, we find that $\Delta H°/N$ is 975 ± 50 cal/(residue mole), and that $\Delta S°/N$ is 2.67 ± 0.1 cal/(residue mole deg). We find that there is no measurable effect of polymer concentration on these thermodynamic parameters and conclude that nonequilibrium aggregation is not present. It is demonstrated that, for poly-L-glutamic acid, three independent measures of helix content agree, namely ultraviolet absorption, titration, and optical rotatory dispersion. The helix content of un-ionized poly-DL-glutamic acid is estimated, from its extinction coefficient at 200 mμ, to be 62%. The implications of these experiments for molecular theories of the conformation of polyamino acids in aqueous solutions are examined. It is found that assignment of the contribution of individual molecular forces (*e.g.*, hydrogen bonding, hydrophobic bonding) to the over-all free energy of transition, whether that assignment is semiemprical or *a priori*, will have to be made with considerably more precision than has thus far been possible, if such theories are to be meaningful.

In view of the pervasiveness of the α-helical conformation in proteins and polypeptides, and of the several theoretical attempts to explain its stability, careful measurements of this stability, and of its entropic and enthalpic composition for a particular case, seem desirable. We present such measurements in this paper. In a sense, the helix–coil transition itself has been rather successfully treated theoretically. The statistical mechanical theories formulated by Peller[3] and by Zimm and Bragg[4] have added rigor to Schellman's suggestion[5] that the helix–coil transition of proteins and polypeptides must be somewhat cooperative. Among the many factors which must contribute to helix stability, however, only the cooperativeness has been elucidated. The fundamental interactions which lead to helix stability are simply lumped and put into the theories as parameters; despite numerous experimental and theo-

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