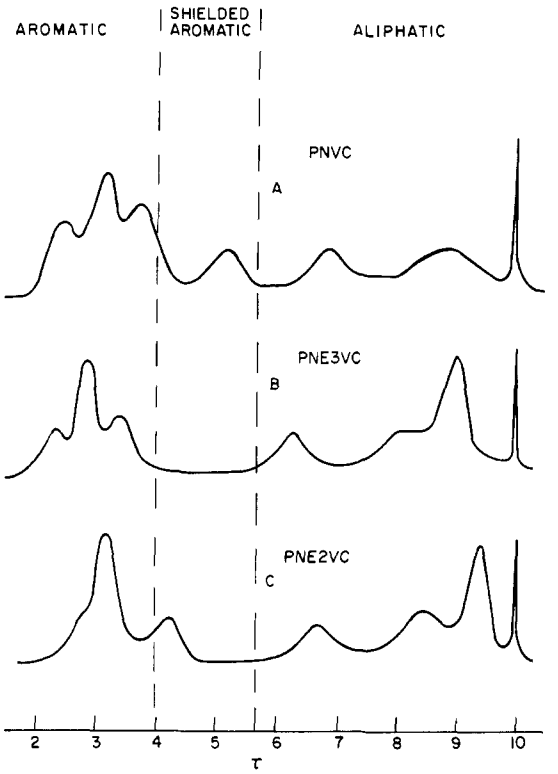


Figure 1. 60-MHz proton nmr spectrum of PNVC, PNE3VC, and PNE2VC at room temperature in $CDCl_3$.

Table I
Chemical Shifts (τ) and Approximate Numbers of Protons

	Aromatic	Shielded Aromatic	Aliphatic
PNVC	2.4, 7.2, 7.8 (~1)	5.0 (~1)	6.8, 8.6
PNE2VC	2.7, 3.1 (~6)	4.1 (~1)	6.7, 8.3, 9.4 (5)
PNE3VC	2.3, 2.7, 3.4 (7)		6.3, 8.0, 9.0 (5)

according to the method of Ledwith.[5] The polymer was precipitated by methanol: M_n 19,400, M_{WD} 2.13.

Poly(*N*-vinylcarbazole) was obtained commercially, and purified repeatedly from H_4furan with methanol: M_n 202,000, M_{WD} 5.1.

The 60-MHz room-temperature proton nmr spectra of PNVC, PNE2VC, and PNE3VC are shown in Figure 1. The various peaks in the proton nmr spectrum are arbitrarily grouped into three regions: aromatic, shielded aromatic, and aliphatic. The assignment of the peaks and numbers of protons are given in Table I. Because of the broadness of the peaks it is difficult to assign individual peaks to their corresponding protons in the molecules. It is possible, however, to distinguish aliphatic from aromatic protons and also to estimate the number of protons in the region designated as shielded aromatic. For PNVC and PNE2VC there is approximately one proton in this shielded aromatic proton region and for PNE3VC none. The intense peak at τ 9.0 in PNE3VC and τ 9.4 in PNE2VC is easily identified as belonging to the methyl group in the *N*-ethyl substituent. These differences in chemical shifts are very significant and will be interpreted below.

In an earlier detailed study of the nmr spectrum of PNVC[3] it was shown that at 220 MHz the shielded aromatic peak could be resolved into a number of peaks whose relative intensities appeared to be temperature dependent. This multiplicity could possibly have been due to contributions from several different positions in the molecule in the different stereochemical sequences and reflect the unusually high steric and spacial constraint in that polymeric environment resulting in large magnetic anisotropies. A further confirmation of this interpretation was obtained when the nmr spectra of a series of *N*-vinylcarbazole–ethyl acrylate, copolymers, were examined. It was shown that the shielded aromatic peak gradually disappeared as the vinylcarbazole concentration was changed from 84 to 54 mol % (Figure 4).[6] For a 1:1 copolymer the nmr spectrum of the aromatic region was very similar to *N*-isopropylcarbazole (Figure 5),[6] where no such steric constraints are possible.

The variations in the degree of shielding in the different polymers contain significant information on the conformation and overall structure of the polymer chain. PNVC appears to represent the polymeric environment in which the carbazole rings are the most tightly packed of any of the carbazyl group containing polymers. PNE2VC appears to be less tightly packed than PNVC but much more so than PNE3VC. On this basis it would seem likely that PNVC should exhibit the largest electronic mobilities and PNE3VC the least. If this correlation can be verified through mobility studies an important relationship between the tertiary structure of the polymer and the electronic properties has been obtained.

(5) P. Hyde, L. J. Kricka, and A. Ledwith, *Polymer*, **14**, 124 (1973).

(6) See ref 3.

Spectrophotometric Detection of Acylimidazole Poly(ethylenimine), an Intermediate in Catalytic Hydrolysis

TIMOTHY W. JOHNSON and IRVING M. KLOTZ*

Department of Chemistry, Northwestern University, Evanston, Illinois 60201. *Received April 13, 1973*

In a previous publication[1] we described derivatives of poly(ethylenimine) (PEI) with catalytic activity toward hydrolysis of nitrophenyl esters. In these derivatives hydrocarbon residues attached to PEI served as binding groups and imidazole residues provided potential catalytic groups. The hydrolytic rate curves in the presence of these polymers indicated that the nitrophenyl esters were cleaved in a two-step kinetic mechanism. Following a fast preequilibrium binding, the first kinetic step was attributed to acylation by substrate of the polymer imidazole residue, accompanied by simultaneous release of nitrophenol(ate). The succeeding kinetic step was ascribed to hydrolysis of the acylimidazole leading to carboxylate ion and regenerated imidazole.

It has seemed desirable to try to detect the postulated acylimidazole intermediate by spectroscopic probing. Acetylimidazole has been shown[2] to have an absorbance maximum at 245 nm with an extinction coefficient of 3000. This absorbance should provide a basis for detection of the intermediate. However, in practice the strong absorbances due to the aromatic ester substrate (nitrophenyl caproate) and the product (nitrophenol(ate)), added to the light scattering from the polymer, have made the spectrophotometric observation of the acylimidazole intermediate unfeasible under the reaction conditions previously described.[1]

(1) I. M. Klotz, G. P. Royer, and I. S. Scarpa, *Proc. Nat. Acad. Sci. U. S.*, **68**, 263 (1971).

(2) E. R. Stadtman in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, Ed., John Hopkins Press, Baltimore, Md., 1954, p 581.

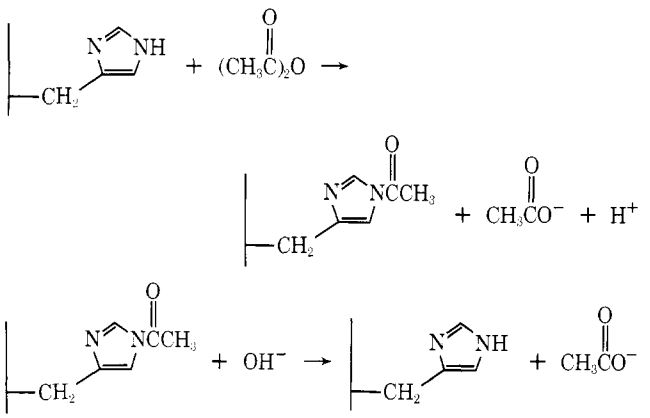
Table I
Formation and Decomposition of Acylimidazoles at 25°

Substrate	pH	Buffer[a]	k (min^{-1}) Anhydride[c]	k (min^{-1}) Laurylacyl-imidazole PEI[d]	k (min^{-1}) Acetyl-imidazole[e]	Max. A Change[b] $A_{max} - A_\infty$
Acetic anhydride	4.5	Acetate	0.13	0.075	0.30	0.08
Acetic anhydride	6.0	Cacodylate	1.0	0.14	0.016	0.10
Acetic anhydride	7.0	Hepes	0.09	0.11	0.008	0.20
Acetic anhydride	7.8	Tris	1.0	0.24	0.018	0.10
Acetic anhydride	8.6	Tris	2.3	0.35	0.09	0.045
Propionic anhydride	6.0	Cacodylate	0.43	0.10		0.22
Propionic anhydride	7.0	Hepes	0.09	0.10		0.30

[a] All buffers are 0.02 M. Hepes = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Tris = tris(hydroxymethyl)aminomethane. [b] Initial substrate concentration = 0.5×10^{-3} M. [c] Spontaneous hydrolysis rate constants which may reflect catalysis by buffer species (see A. R. Butler and V. Gold, *J. Chem. Soc.*, 2305 (1961)). [d] From exponential decay of spectrophotometrically observed intermediates. See text and Figure 1. [e] From ref 3.

We have now circumvented these difficulties by using a weakly absorbing substrate, acetic anhydride, in place of the nitrophenyl esters.

When acetic anhydride and laurylimidazole PEI are mixed the following reactions (in addition to spontaneous hydrolysis) are postulated to occur



If they do, and if the second reaction is rate limiting, the concentration of laurylacetylimidazole PEI should first rise rapidly, then reach a maximum, and finally decrease. Since the acylimidazole intermediate is the only species which absorbs strongly at 245 nm, the absorbance at this wavelength should follow the same pattern.

When acetic anhydride at concentrations of from 0.5×10^{-3} to 4.0×10^{-3} M is allowed to react with laurylimidazole PEI, with imidazole concentrations of 1.3×10^{-4} to 1.7×10^{-4} M, the absorbance follows the predicted bell-shaped profile. To obtain more accurate values for the acetylimidazole concentrations, the observed absorbance readings were corrected for the small contribution from residual acetic anhydride. These corrections were simply the absorbances of a similiar solution containing only substrate and buffer. Absorbances of acetic anhydride decay exponentially with rate constants are indicated in the fourth column of Table I. Early in the reaction the correction should be rather accurate, and, in any case, it is small. Later in the reaction the correction is very small.

The corrected absorbances (shown in Figure 1), which have the same general form as the uncorrected curves, follow a time course in accord with the proposed two-step mechanism. The initial rates at pH 4.5 (and also those at pH 8.6) indicate a greater than zero-order dependence on the acetic anhydride concentration. The maximum absorbances at pH 4.5 (and at pH 8.6) increase approximately twofold when initial anhydride concentration is increased from 0.5×10^{-3} to 1.0×10^{-3} M. The maximum absorbance change possible in these experiments, assuming complete conversion of the imidazole to acetylimidazole, is 0.5. The observed absorbance changes of 0.045–0.30, shown in the last column of Table I, imply that conversions of about 10–60% are actually attained. Shortly after the time of maximum absorbance in each experiment, the decay of the intermediate becomes exponential and remains so over at least three half-lives. The first-order rate constants for the decay are listed in Table I along with the rate constants for hydrolysis of acetylimidazole.[3] The rate of deacylation on the polymer increases with pH over the range studied, in contrast with simple acetylimidazole, which displays a rate minimum at about pH 7. The behavior of the polymer is not surprising, however, since the charge on the polymer will suppress protonation of the acetylimidazole attached to it. An acylimidazolium ion, the species responsible for the increase in rates at low pH,[3] is thus formed to a lesser extent on the polymer than in the small model compound. At higher pH the cationic charge on the polymer increases the concentration of hydroxyl ion in the vicinity of the attached acetylimidazole groups and hence rates of deacylation are enhanced relative to those of small molecules.

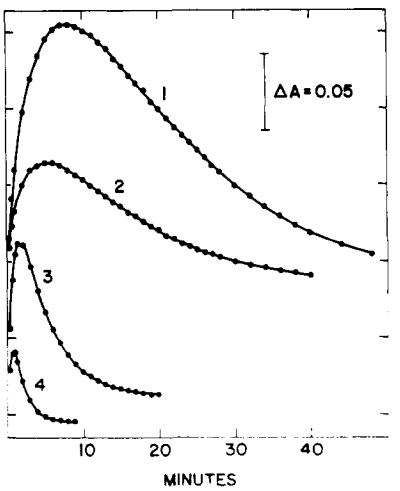


Figure 1. Formation and subsequent decomposition of an acetylimidazole intermediate in aqueous solutions of laurylimidazole PEI ([imidazole] = 1.7×10^{-4} M) and acetic anhydride. Initial concentrations of acetic anhydride and pH values are: 1, 1.0×10^{-3} M, pH 4.5; 2, 0.5×10^{-3} M, pH 4.5; 3, 0.5×10^{-3} M, pH 7.8; and 4, 0.5×10^{-3} M, pH 8.6.

In the previous publication[1] the rate constant for hydrolysis of laurylcaproylimidazole PEI to caproate ion and

(3) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, 234, 1272 (1959).

laurylimidazole PEI was estimated to be 0.06 min^{-1} at pH 7.3. In the present study we have found the rate constant for hydrolysis of laurylacetylimidazole PEI to acetate ion and laurylimidazole PEI under similar conditions to be 0.1–0.2 min^{-1}. In the earlier study, the hydrolysis rate was inferred by an indirect method from the turnover rate in a steady-state situation. In view of the uncertainties in the indirect method and the difference in size of the acyl group in the two cases, the approximate equality of the deacylation rate constants in gratifying.

To resolve the uncertainty due to the difference between acetate and caproate, an experiment was attempted using caproic anhydride as the substrate. The reaction could not be followed, however, presumably because of the low solubility of caproic anhydride. On the other hand, with propionic anhydride as substrate, in which case there is no solubility problem, the course of the reaction was very much the same as in the experiments with acetic anhydride. The rate constants for decomposition of propionylimidazole are also reported in the table. They are strikingly similar to those for acetylimidazole.

To summarize, we have spectrophotometrically observed an acylimidazole intermediate in a reaction analogous to that for which kinetic analysis had previously suggested such an intermediate. The kinetic parameters deduced from indirect spectrophotometry are nearly the same as those inferred from indirect measurements described in the earlier work.[1]

Acknowledgment. This investigation was supported in part by a grant from the National Science Foundation.

Vacuum Ultraviolet Circular Dichroism of Poly(L-alanine) Films

MICHAEL A. YOUNG and E. S. PYSH*

Department of Chemistry, Brown University, Providence, Rhode Island 02912. *Received May 23, 1973*

There has been a substantial advance in the quantum mechanical description of polymer optical properties, both in the development of powerful and efficient formalisms, and in the completion of detailed spectral calculations on several polymer systems.[1] The calculations are essentially low-frequency limit applications of theory because experimental data describing the monomer electronic states are good only for those states with low excitation energy. It is therefore important to determine at what energy the polymer calculations break down from the lack of data describing the high-energy states. Calculations of the circular dichroism of polypeptide α helices, for example, are accurate to 190 nm, which is near the limit of the energy range experimentally accessible on commercial spectrometers. Our purpose here is to show that for poly(L-alanine), which contains only saturated C–H bonds in its side chain and which is therefore a good model polymer, the calculations break down just beyond 190 nm.

Our circular dichroism instrument consists of a hydrogen discharge cold-cathode light source, a 1-m vacuum ultraviolet monochromator, a biotite linear polarizer, a stressed-plate CaF_2 modulator as quarter-wave retarder,[2] and a photomultiplier with sodium salicylate coated window. The light intensity reaching the photomultiplier when there is no sample in the light path determines a dc signal which, in our instrument, is maintained at a constant level. The light intensity reaching the photomultiplier when there is an optically active sample in the path determines an ac signal which in our instrument is proportional to the circular dichroism. After a proper one-time calibration circular dichroism can be recorded directly. The voltage on the stress plate modulator is programmed by mechanical linkage to the monochromator wavelength drive to maintain quarter-wave retardation. Our instrument is limited in wavelength range only by the transmission properties of the CaF_2 of the modulator. Other details of our instrument will be reported separately. A similar instrument has been described by Johnson,[3] and Schnepp[4] has described an early version of an instrument with moderate differences.

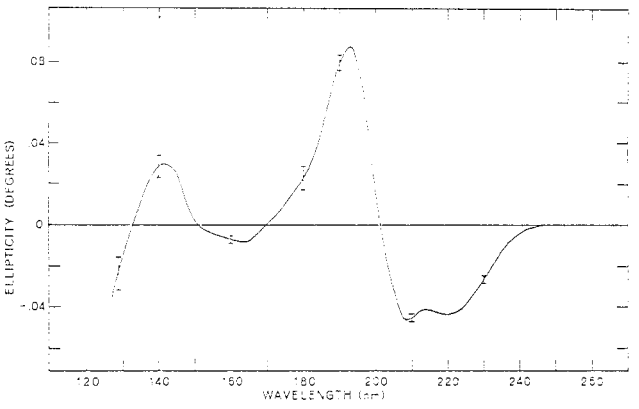


Figure 1. Circular dichroism of α-helical poly(L-alanine).

Poly(L-alanine) (Pilot Chemical Co.) was dissolved in trifluoroethanol in which it takes up the alpha helical conformation.[5] Films were prepared in a nitrogen-flushed glove box by evaporation to dryness onto calcium fluoride disks of 1 mm thickness. The total absorbance of sample plus CaF_2 was less than 1 at $\lambda > 1500$ Å; the absorbance rose below 1500 Å to approximately 2 at 1300 Å. The film thickness was estimated to be approximately 1800 Å. Spectra were recorded with a spectral slitwidth of 1.6 nm using a time constant of 10 sec. and a scan rate of 1 nm/min.

It is known that the circular dichroism of some polymer films show birefringence effects, in that the signal obtained with such films is observed to depend on the orientation of the film in the light path. We observed such an effect on some of our films. Figure 1 shows the circular dichroism obtained with a film which exhibited no orientation dependence. In this work our instrument was calibrated by measuring the circular dichroism of the same film using a Cary 60-6001 spectropolarimeter in the range 190–240 nm.

Figure 1, in the region from 190 to 240 nm, shows the circular dichroism which is characteristic of a polypeptide α helix. This part of the spectrum can be reproduced correctly with detailed quantum mechanical calculations which include the coupling of the lowest two transitions of the monomeric peptide chromophores. These same calculations however give a negative circular dichroism near 180 nm, which has until recently been outside the range of experimental observations. From Figure 1 it can be concluded that this feature is not observed experimentally. Johnson and Tinoco[6] reported a similar conclusion from

(1) See for an example and for additional references E. S. Pysh, *J. Chem. Phys.*, **52**, 4723 (1970).
(2) (a) Purchased from Morvue Electronic Systems, Tigard, Oregon. (b) J. C. Kemp, *J. Opt. Soc. Amer.*, **59**, 950 (1969).
(3) W. C. Johnson, Jr., *Rev. Sci. Instrum.*, **42**, 1283 (1971).
(4) O. Schnepp, Susan Allen, and Earl Pearson, *Rev. Sci. Instrum.*, **41**, 1136 (1970).
(5) F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.*, **90**, 2755 (1968).
(6) W. C. Johnson, Jr., and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **94**, 4389 (1972).