Nevertheless, until an independent determination of one of these three parameters has been made for this particular system, these values must be regarded as quite tentative.

The ultrasonic data indicate that the helical content of the chain is increasing above pH 11.4, although some divergence from the theoretical curves is observed. However, the circular dichroism data suggest that the amount of helix tends to reach a limiting value at this pH. Hydrolysis of the peptide chain above pH 11.85 does not allow accurate ultrasonic data to be obtained above this pH to see if a helical fraction greater than $\sim$0.7 is predicted by the ultrasonic data. Also if θ is obtained from the molar ellipticity by the use of the empirical equations used by Yang,[10,16] the predicted maximum in τ and μ_m occurs at pH 10.95 rather than 11.25 as inferred from the ultrasonic data. However, the uncertainty involved in using such empirical equations in nonaqueous solvents has already been pointed out. No ready explanation of these discrepancies exists, although the two techniques certainly must measure different properties of the polymer.

In the region of pH 9 a small chemical relaxation still exists. The amplitude of this relaxation is far smaller than the amplitudes observed nearer the transition midpoint, but is larger than predicted by an extrapolated value of θ and eq 5 and 6. This may indicate the existence of a small amount of helical content even at high degrees of protonation of the polypeptide side chains, or may be due to the fact that some of the theoretical equations used are not valid far from the midpoint of the transition.

In summary, ultrasonic attenuation measurements of poly-L-ornithine appear to measure directly the dynamics of the helix–coil transition; moreover the pH dependence of the relaxation time and the amplitude of the relaxation process are in quantitative agreement with the theoretical predictions. The rates associated with this polypeptide helix–coil transition can be regarded as approximate upper bounds for the rates of similar, but usually more complex, cooperative processes in proteins.

Conformational Aspects of Polypeptide Structure. XXX. Rotatory Properties of Cyclic and Bicyclic Amides. Restricted and Rigid Model Compounds for Peptide Chromophores[1]

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Abstract: The optical properties of monocyclic and bicyclic amides have been examined by optical rotatory dispersion (ORD) and circular dichroism (CD) techniques. The signs of the rotational strength of the n–π^* transitions of L-α-aminocaprolactam and L-pyrrolid-2-one-5-carboxylic acid are interpreted in terms of both quadrant and octant rules. The effect of charged side-chain groups on the amide chromophores is also discussed. In addition to n–π^* and π–π^* Cotton effects, a third band in the spectra of bicyclic lactams has been observed. Infrared and circular dichroism dilution studies demonstrate that this Cotton effect arises from association of the lactams to form dimers. We have also been able to separate solvent and conformational effects on the ORD and CD spectra for the completely rigid bicyclic amides since alterations in conformation for these compounds can not occur.

Current attention of far-ultraviolet optical rotatory dispersion (ORD) and circular dichroism (CD) for polypeptides and proteins arises from substantial improvements in instrumentation.[4] Recent review articles include discussions on the nature of electronic transitions in the peptide (amide) chromophore.[5–10] We and others[5,6,9,10] believe that the clearest approach to assignment of the amide transitions comes from using low molecular weight optically active compounds. A limited number of such studies has appeared.[9–18]

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(3) Submitted by Joseph Falcetta to the faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the Ph.D. in Chemistry.

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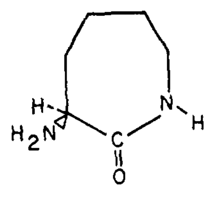
In this context we undertook to examine the optical rotatory properties of four lactams: L-α-aminocaprolactam (I); L-pyrrolid-2-one-5-carboxylic acid [pyroglutamic acid] (II); 1,7,7-trimethyl-3-azabicyclo[2.2.1]heptan-2-one (III); and 1,7,7-trimethyl-2-azabicyclo[2.2.1]heptan-3-one (IV).



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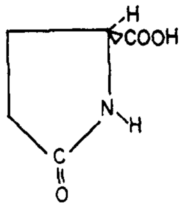

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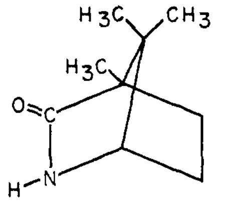

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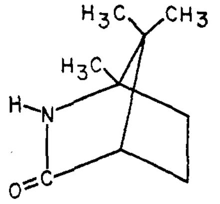

IV

The first two materials are monocyclic, while the second two are bicyclic and thus hold the amide chromophore in a completely rigid geometry.

Recently, the isotropic absorption spectra of a number of primary, secondary, tertiary, cyclic, and acyclic amides have been extensively examined by Schellman.[11] He has shown a general red shift for the intramolecular charge transfer $\pi-\pi^*$ transition of the amide chromophore in going from cyclohexane to water. The shift results from the favorable interaction of the highly polar excited state with the solvent cage of water. Since this effect is negligible with tertiary amides, solvent interaction with the nitrogen atom, *via* its attached hydrogen must be invoked. Even if the amide has the *cis* conformation, lactams show spectral properties similar to secondary amides. In nonpolar organic solvents, such as cyclohexane, these compounds exhibit a shoulder in their ultraviolet spectra in the 225–235-mμ region, which has been assigned to the $n-\pi^*$ transition of the amide chromophore. In water, because of the blue shift of the $n-\pi^*$ transition and the red shift of the $\pi-\pi^*$ transition, the shoulder in the long-wavelength side of the 190-mμ main band disappears.

Circular dichroism and optical rotatory studies of diamides,[12] lactams,[9,10,13–16] and related compounds, such as spirobihydantoin[17] and diketopiperazines[9,12,16,18] have been reported. Five bands are present in the circular dichroism spectrum of (−)-spirobihydantoin in water, ethanol, and tetrahydrofuran.[17] The two bands at longer wavelengths have been assigned to the $n-\pi^*$ transitions, the last three to the $\pi-\pi^*$ transitions of the chromophores in the molecule.

Balasubramanian,[18] Schellman,[12] Urry,[9] and Fasman[16] have independently studied the ORD and CD of diketopiperazines. In early 1965 Wolf published the optical rotatory dispersion and circular dichroism spectra of two steroid ∂-lactams.[14] A positive Cotton effect at approximately 215 mμ was observed in methanol. In the optical rotatory dispersion spectrum the beginning of a negative Cotton effect, centered well below 200 mμ, is also apparent. In order to obtain further information on the difficult to observe $n-\pi^*$ amide band, Schellman and his associates[13] studied the ORD of L-3-aminopyrrolid-2-one. They assign the observed 230–235-mμ small positive Cotton effect in dioxane and acetonitrile to the $n-\pi^*$ transition of the amide chromophore. The beginning of a much larger negative Cotton effect, possibly related to the amide $\pi-\pi^*$ transition, is also discernible. In water the former Cotton effect disappears, probably because it is completely obscured by the latter band. In less polar organic solvents the blue shift of the $\pi-\pi^*$ transition and the red shift of the $n-\pi^*$ transition are sufficient to allow partial resolution of these Cotton effects. Schellman was the first to report an $n-\pi^*$ Cotton effect of the amide group for substances simpler than polypeptides and proteins.[13] It was concluded that the peptide $n-\pi^*$ Cotton effect observed in L-3-aminopyrrolidone as well as in helical polypeptides results from a rigid conformation of properly distributed atoms in an environment of low dielectric constant.

Results and Discussion

In our studies of mono- and bicyclic amides we seek to determine the effect of conformational rigidity on the ORD and CD spectra of compounds containing the amide chromophore. The four compounds examined contain *cis*-amide structures. However, as noted above, no spectral differences have been predicted between *trans*- and *cis*-amide chromophores.[13]

L-α-Aminocaprolactam (I). The ORD spectra of L-α-aminocaprolactam are shown in Figure 1. A negative Cotton effect with a trough at 220 mμ is apparent in water and in trimethylphosphate. The spectra in pure water and in aqueous buffers at a pH of 2 and 9 show similar molar rotation values and locations of the troughs. In dioxane, on the other hand, an additional positive longer wavelength Cotton effect is observed. The exact location of the peak cannot be established because of overlapping of the negative Cotton effect. These results agree with those reported by Schellman for L-α-aminopyrrolid-2-one.[13]

We obtained a much clearer picture of the rotatory properties of L-α-aminocaprolactam using circular

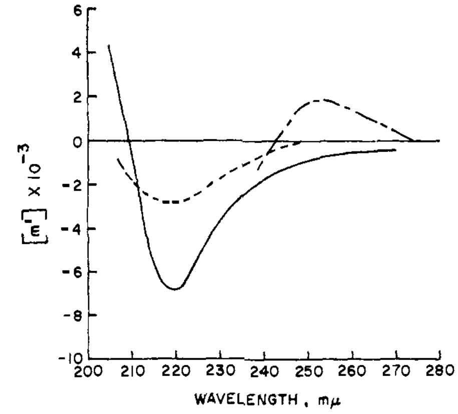

Figure 1. ORD spectra of L-α-aminocaprolactam in water (———) trimethyl phosphate (— — —), and dioxane (—·—).

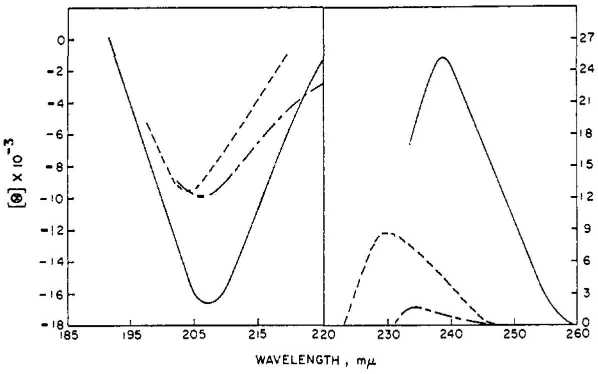

Figure 2. CD spectra of L-α-aminocaprolactam in dioxane (———), acetonitrile (— — —), water (—·—), (260–220 mμ); in water (———), trimethyl phosphate (—·—), and acetonitrile (— — —) (220–185 mμ).

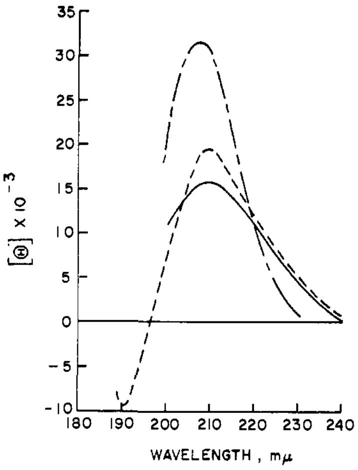

Figure 3. CD spectra of L-pyroglutamic acid in pure water (— — —) and in aqueous solutions at pH 2 (———) and pH 9 (—·—).

dichroism. Figure 2 shows a positive Cotton effect in dioxane, acetonitrile, and water. It is impossible to assign the exact location of the weak band in water because of the large adjacent negative Cotton effect. However, the location of the band and its blue shift in going from dioxane to acetonitrile leads us to assign this positive Cotton effect to an n–π* transition in agreement with Schellman, who made a similar assignment based on his ORD results for the five-membered ring homolog.[13]

The assignment of the negative Cotton effects, shown in Figure 2, is consistent with a π–π* transition, since the band is blue shifted in going from water (207 mμ) to less polar solvents, such as trimethylphosphate and acetonitrile (205 mμ). Nevertheless, we must point out that the location of these negative Cotton effects in the CD spectra is greatly displaced toward the red as compared with normal π–π* bands. There is a possibility that this band arises in part from an n–σ* transition, which has recently been observed in the uv spectra of amides.[19] The uv spectrum appears to support the latter possibility, since it exhibits a large maximum at 189 mμ with a shoulder in the 205–210-mμ region. On the basis of quadrant and octant rules, Schellman[13,20] and Urry[9] predict a positive Cotton effect for the n–π* transition of L-α-aminopyrrolid-2-one. In fact the amino group is in the $+x$, $-y$, and $+z$ octant (or in the $+x$ and $-y$ quadrant) such that the product of direction cosines is negative in the expression of Caldwell and Eyring for the rotational strength of an n–π* transition. The coefficient is positive because of the negative sign preceding the equation. Our results on L-α-aminocaprolactam are completely in agreement with the above interpretation.

L-Pyrrolid-2-one-5-carboxylic Acid [L-Pyroglutamic Acid] (II). We have also examined the ORD and CD spectra of L-pyroglutamic acid (compound II) in a number of solvents of different polarity to investigate the characteristics of n–π* and π–π* transitions. This compound contains the amide and carboxyl chromophores. In a sense, compound II is similar to compound I in that both have groups adjacent to the amide chromophore which can vary their effects as a function of ionization. As noted above, we do not observe any significant influence of protonation of the amino group on the optical rotatory properties of compound I. Figure 3 shows the CD of compound II in water and the substantial influence of pH on the intensity and location of the positive CD band. Pyroglutamic acid has been shown to be a strong acid.[21] Hence we note the similarity between the pyroglutamic acid measured at pH 2 and in pure water.

Saidel[22] studied the effect of ionized and un-ionized carboxyl groups on the isotropic absorption spectrum of the peptide chromophore. By studying the ultraviolet spectra of a number of compounds containing carboxyl functions attached to the same carbon atom as the peptide nitrogen, he demonstrated that an un-ionized carboxyl group interacts with the peptide group to produce an observable transition. The ionization of the carboxyl group affects this interaction altering the isotropic absorption spectra of these compounds. The maximum effect is observed when the two groups are attached to the same carbon atom. Saidel noted that steric effects cannot be invoked to explain the alteration in absorption spectra, since L-pyroglutamic acid exhibits the same spectral changes upon ionization of the carboxyl group as peptides.

In 1965 Legrand and Viennet[23] demonstrated by CD measurements that the carboxyl groups shows a band at 217 mμ (Δε 3.26) in the un-ionized form and a band at 210 mμ (Δε 2.22) in the ionized form. The ionization of the carboxyl group appears to cause a blue shift and a marked decrease in ellipticity of the n–π* transition of simple optically active acids, perhaps because of the greater symmetry of the carboxylate ion. We find an increase in intensity and a blue shift for the CD of compound II in going from pH 2 to pH 9. This is different from the effect noted by Legrand and Viennet.[23] To explain our results we must invoke an auxochromic effect of the carboxylate anion on the amide chromophore. Our circular dichroism study

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(Figure 3) demonstrates that L-pyroglutamic acid in water exhibits two Cotton effects in the far ultraviolet region, the first positive and centered at about 210 mμ, the second negative and centered at 191 mμ, which we assign to the $n-\pi^*$ and $\pi-\pi^*$ transitions of the two chromophores in the molecule. Other possibilities, including electric-magnetic coupling of the $n-\pi^*$ transition of one chromophore with the $\pi-\pi^*$ transition of the other chromophore[20] can be invoked. Table I shows that the long-wavelength positive Cotton effect undergoes a red shift with decreasing solvent polarity, as expected for an $n-\pi^*$ transition. The ellipticity values for compound II are approximately the same in all the solvents studied except dioxane where a decrease of about 60% is observed. If water is replaced by mixtures containing different percentages of dioxane the red shift of the $n-\pi^*$ transition parallels the decrease in the ellipticity value. At this time we can offer no explanation for the observed decrease in ellipticity of this band.

Table I. Location and Molar Ellipticity Values of Amide $n-\pi^*$ Transition of L-Pyrrolid-2-one-5-carboxylic Acid in Different Solvents

Solvent	λ, mμ	$[\theta] \times 10^{-3}$
Hexafluoroacetone trihydrate	209	+18.5
Hexafluoroisopropyl alcohol	209.5	+20.7
Water	210	+19.5
Water-methanol (1:1)	211	+16.4
Water-trimethyl phosphate (1:1)	213.5	+20.0
Water–dioxane (1:1)	214	+16.2
Methanol	216	+17.2
Trimethyl phosphate	219.5	+17.3
Dioxane	223	+ 6.8

A most dramatic alteration of the CD spectrum of compound II is encountered when studies are carried out in 70% perchloric acid. We find (Figure 4) a broad positive ellipticity with a hint of a shoulder between 210 and 200 mμ. Bovey[10] noted that the sign of the $\pi-\pi^*$ Cotton effect for simple model compounds is reversed in very strong acid solvents. Our results agree with this finding. We are not able, however, to decide if the $n-\pi^*$ transition is completely repressed because of the shoulder noted above.

In his review article on optical rotation Urry[9] examined the CD of L-pyroglutamic acid at pH 1.1 and 7 and in trifluoroethanol, as part of a study of L-pyrrolid-2-ones with different substituents in position 5. Two Cotton effects, the first positive at 207–212 mμ and the second negative at 187–192 mμ were observed in all the three solvents. On the basis of the shifts of the position of the bands with solvent polarity and of their anisotropy values he assigned the two Cotton effects to the $n-\pi^*$ and $\pi-\pi^*$ transitions, respectively. Upon ionization of the carboxyl side-chain group a blue shift of about 5 mμ and a twofold enhancement of ellipticity values were observed for both bands.

The quadrant[13,20] and octant rules[9] predict a negative Cotton effect for the $n-\pi^*$ transition of compound II. The fact that we observe a positive Cotton effect may be explained by the static charge term in the expression of the rotational strength of the $n-\pi^*$ transition for a single chromophore and perturbing group.[9] Urry has shown that the more electronegative the vicinal group in position 5 of the pyrrolidone ring or the greater the net negative charge on this group, the greater is the positive rotational strength.[15] The sign of the band, which is negative for 5-iodomethyl-, 5-methyl-, and 5-hydroxymethylpyrrolid-2-ones becomes positive in the case of 5-carboxy derivatives. This is excellent support for the idea of the importance of charge and electronegativity in determining magnitude and sign of rotational strength.

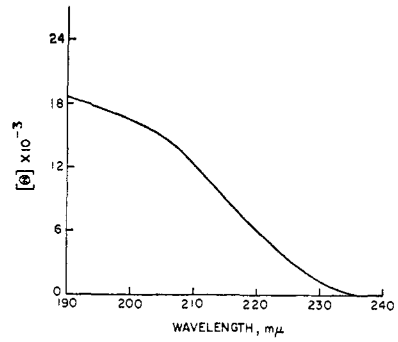

Figure 4. CD spectrum of L-pyroglutamic acid in 70% perchloric acid.

Camphorolactams. 1,7,7-Trimethyl-3-azabicyclo[2.2.1]heptan-2-one (III) and 1,7,7-Trimethyl-2-azabicyclo[2.2.1]heptan-3-one (IV). By placing the amide chromophore in a ring (compounds I and II) we achieve some conformational restrictions. However, we cannot rule out conformational effects on the rotatory properties of these materials, as the temperature and solvents are changed. In order to eliminate all conformational variations we prepared two bicyclic lactams (III and IV) and studied their rotatory properties.

The lactams (1,7,7-trimethyl-3-azabicyclo[2.2.1]heptan-2-one and 1,7,7-trimethyl-2-azabicyclo[2.2.1]heptan-3-one) were synthesized by known procedures commencing with *d*-camphoric anhydride.[24,25] The final products (III and IV) contain two asymmetric centers, which fortunately do not complicate our analysis since the molecules are composed of inherently symmetric chromophores asymmetrically perturbed. The chirality of the asymmetric centers are not altered from compound III to compound IV. The fact that ORD and CD spectra of compounds III and IV are almost mirror images of each other can be interpreted by a change of the chirality of the amide chromophore with respect to the perturbing asymmetric centers.

We carried out optical rotatory dispersion studies on both lactams III and IV in different solvents (Figures 5 and 6). The magnitude of the Cotton effect is in the range found for helical poly-α-amino acids.[7] This finding is in sharp contrast to monocyclic lactams,[12] where the intensities of the Cotton effects are about 20% of the values found for helical poly-α-amino acids. This enhancement is undoubtedly a function of the rigid structure of these bicyclic compounds.

We compared the ORD spectra of compound IV in water and 8 *M* urea and found little difference. These results provide evidence that the changes in ORD of many proteins in these solvents[5,26] come from

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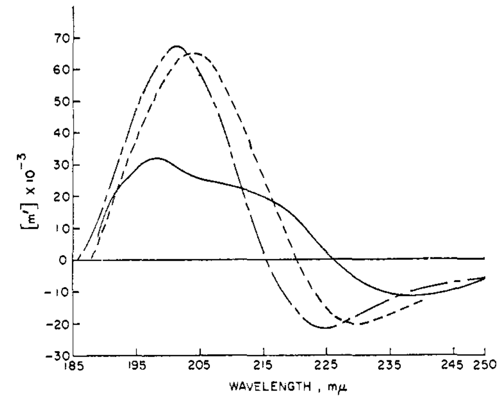

Figure 5. ORD spectra of camphorolactam III in trifluoroethanol (—·—), ethanol (— — —), and hexane (———).

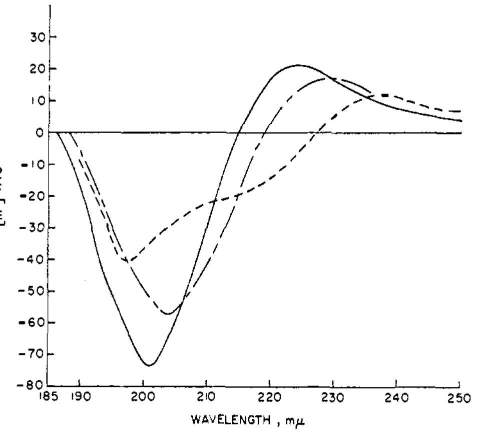

Figure 6. ORD spectra of camphorolactam IV in trifluoroethanol (———), ethanol (—·—), and hexane (— — —).

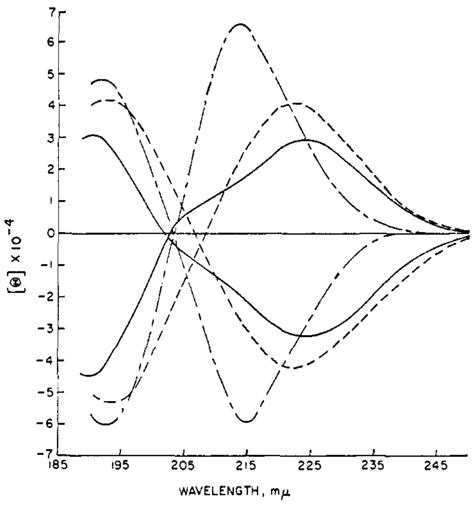

Figure 7. CD spectra of camphorolactams III (high wavelengths show negative bands) and IV (high wavelengths show positive bonds) in hexane (———), acetonitrile (— — —), and water (—·—).

conformational changes for the high molecular weight compounds rather than from a direct effect caused by the denaturing agent on the peptide chromophore. For both lactams there is a significant red shift of the higher wavelength extremum on going from water to nonpolar solvents. On the basis of location and shifts with solvent polarity we assign this Cotton effect to the n–π* transition of the amide chromophore. An extensive shoulder appears on the short-wavelength side of the 225-mμ extremum in hexane. (We shall discuss the nature of this effect on the basis of our CD data.) The beginning of a larger low-wavelength Cotton effect, probably related to the π–π* transition of the amide chromophore, is also apparent in the ORD spectra of these camphorolactams (III and IV).

Circular dichroism spectra of the two lactams were obtained in water, acetonitrile and hexane (Figure 7). Since the spectra of the two lactams are essentially mirror images (Figure 7) as noted above, the following discussion is focused primarily on the spectra of the lactam IV.

The positive band undergoes a red shift on changing the solvent from water to acetonitrile to hexane (214 to 222 to 224 mμ) and the molar ellipticity values markedly decrease. We assign this positive Cotton effect to the n–π* transition of the amide chromophore. The low-wavelength negative band experiences a slight blue shift (191.5–189.5 mμ) in going from water to hexane. This shift could be due to a direct influence of the solvents on the chromophore or to differences in degree of association in the various solvents. Significant differences for the molar ellipticity values in the solvents examined are observed also for this band, which is probably associated with the π–π* transition of the amide chromophore. The shoulder apparent in the low-wavelength side of the positive Cotton effect in hexane caused us to investigate whether it arises from association or from a new transition. The possibility of the presence of an n–σ* transition between the n–π* and π–π* transitions of the amide group has recently been suggested from a study of the electronic absorption spectra of a number of amides.[19] The band was observed in primary, secondary, and tertiary amides having both open-chain and cyclic structures. Since the existence of this transition had not previously been considered in the assignment of bands in the circular dichroism spectra of high and low molecular weight compounds containing the amide (peptide) chromophore, it was extremely important to determine the contribution of this transition to the optical activity of these systems. Three very recent studies[9,15,16] indicate the possible contribution of such transition to the CD spectra of compounds containing the amide chromophore.

The CD spectra of lactam (III) is similar to that obtained recently by Urry[15] for L-5-methylpyrrolid-2-one. In cyclohexane this compound exhibits a negative band centered at approximately 220 mμ and a positive band centered below 190 mμ. He assigned these bands to the n–π* and π–π* transitions, respectively. However, there is also a prominent negative band at 202 mμ. A determination of the effect of lactam concentration on the CD spectra clearly demonstrates that this peak arises primarily from association. However, the spectra at the lowest concentration studied (1.83×10^{-4} *M*) still shows a detectable 200 mμ negative band. Urry[15] indicates that this band may be a composite of two bands, one arising from lactam association and the other possibly from an n–σ* transition.

To determine the extent of association of the lactam (IV) in hexane we carried out qualitative infrared determinations (Figure 8) and also ascertained the effect of concentration on the CD spectra (Figure 9). Infrared spectroscopy has frequently been employed to study the dimeric association of lactams in nonpolar solvents.[13,27,28]

Litman and Schellman[13] employed such qualitative infrared experiments to rule out the possibility of association for L-3-aminopyrrolid-2-one in the solvents they studied. In the solid state (KBr pellet) their compound shows a splitting of the carbonyl stretching frequency (1705 and 1694 cm^{-1}). They noted single absorption bands in acetonitrile and dioxane (6×10^{-3} *M*) at 1714 and 1712 cm^{-1}, respectively. The results were interpreted to indicate the presence of both monomer and dimer in the solid state, but only monomer in acetonitrile and dioxane solutions.

Klemperer, *et al.*,[28] observed a splitting of the carbonyl stretching frequency for γ-butyrolactam in carbon tetrachloride solution. The observed values 1716 and 1703 cm^{-1} were assigned to monomer and dimer.

We obtained the infrared spectra of the lactam IV in the solid state and in hexane solutions. The solid sample (KBr pellet) exhibits a single peak for the amide I band at 1700 cm^{-1}. However, in hexane splitting is observed (Figure 8). At a concentration of 2.0 g/l. two peaks are found, at 1735 cm^{-1} and at approximately 1708 cm^{-1}. The lower frequency band is more intense. On dilution of the hexane solution, the 1735-cm^{-1} peak increases in intensity. At a concentration of 0.065 g/l. the 1708-cm^{-1} peak has practically disappeared.

This study demonstrates that the lactam must exist almost exclusively as a dimer in the solid state. However, in hexane solution both dimer and monomer are present until a concentration of less than 0.065 g/l. Below this concentration the predominant species present is monomer.

This is also reflected in our circular dichroism results of the lactam IV at different concentrations (Figure 9). The shape of the spectra changes drastically with dilution, the shoulder disappearing completely at a concentration of 0.029 g/l. (1.9×10^{-4} *M*). Only a slight shift occurs in the location of the positive band from the highest to the lowest concentration.

The results of this study seem to be different from those obtained by Urry,[15] who found that the CD spectra of L-5-methylpyrrolid-2-one in cyclohexane maintains the presence of a second negative band around 200 mμ at the most dilute concentration examined (1.83×10^{-4} *M*).

We conclude from the CD dilution study and the infrared data that the shoulder observed in hexane probably arises from a transition splitting phenomenon brought about by association of the lactam molecule to a dimer. Nevertheless, we cannot completely rule out the possibility of the contribution of the n-σ* transition to the ORD and CD spectra of lactams, since in our completely rigid systems the huge molar ellipticity values of the n-π* and π-π* transitions could have masked the effect of the n-σ* transition.

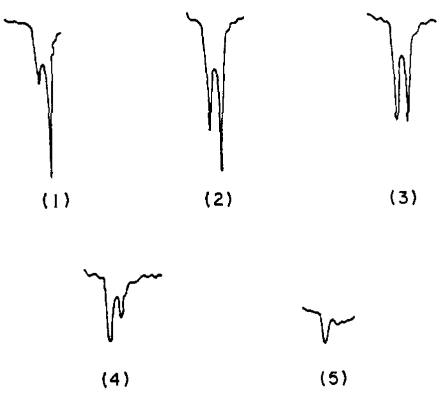

Figure 8. Infrared spectra of amide I bands of camphorolactam IV in hexane solutions (1750–1700 cm^{-1}): (1) 2 g/l., (2) 0.5 g/l., (3) 0.25 g/l., (4) 0.13 g/l., (5) 0.065 g/l.

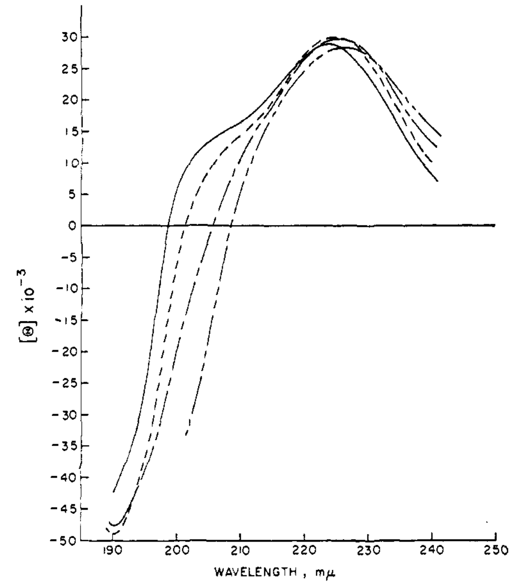

Figure 9. CD spectra of camphorolactam IV in hexane at various concentrations: *c* 8.03 mg/ml (———), *c* 1.61 mg/ml (— — —), *c* 0.24 mg/ml (—·—), and *c* 0.029 mg/ml (—··—).

Experimental Section

Optical rotatory dispersion and circular dichroism spectra were obtained from a Cary ORD 60 automatic recording polarimeter equipped with a Cary prototype CD attachment. A 450-W Osram xenon lamp was employed as the light source. Nmr spectra were measured on a Varian Associates A-60 instrument using tetramethylsilane as an internal reference. A Perkin-Elmer 521 grating infrared spectrophotometer was employed to determine infrared spectra. All melting points are uncorrected.

Solvents for Spectroscopic Studies. Distilled water was employed for all aqueous measurements. Trifluoroethanol was purchased from Halocarbon, Inc. Dioxane, acetonitrile, ethanol, and hexane were spectro grade from either Fisher Scientific Co. or Matheson Coleman and Bell. Trimethyl phosphate (Aldrich Chemical Co.), hexafluoroisopropyl alcohol (Aldrich Chemical Co.), and hexafluoroacetone trihydrate (Hynes Chemical Research) were used without further purification. The latter was stored under refrigeration. Standard buffer solutions pH 2.00 ± 0.02, 25° (potassium chloride–hydrochloric acid buffer), 0.05 *M* and pH 9.00 ± 0.2, 25° (boric acid–potassium chloride–sodium hydroxide buffer), and 0.1 *M* and 70% perchloric acid were purchased from Fisher Scientific Co. and Aldrich Chemical Co., respectively.

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L-α-Aminocaprolactam (I), mp 66–68°, $[\alpha]^{25}\text{D}$ $-34.2°$ (in water), was kindly supplied by Allied Chemical Company (see acknowledgment).

L-Pyrrolid-2-one-5-carboxylic acid (II), mp 154–157°, $[\alpha]^{25}\text{D}$ $-10°$ (in water), was purchased from Aldrich Chemical Co. and recrystallized from hot water.

1,7,7-Trimethyl-3-azabicyclo[2.2.1]heptan-2-one (III) was prepared from aminocamphonanic acid following the procedure of Noyes and Potter.[25] The aminocamphonanic acid precursor was synthesized from camphoric anhydride as described by Noyes.[24] Purification of the lactam was accomplished by sublimation at 110° (0.5 mm); mp 202–203° (lit.[25] mp 203°); $[\alpha]^{26}\text{D}$ $-59.2°$ (0.0978 g/cc, ethanol) (lit.[25] $[\alpha]^{29}\text{D}$ $-60.5°$ (0.1 g/cc, ethanol)].

The nmr spectrum ($CDCl_3$) consisted of a broad band at τ 3.05 (1 H, NH), three singlets at 8.97, 9.03, and 9.12 (9 H, CH_3), an unresolved singlet at 6.67 (1 H, CH), and a number of complex multiplets at 7.9–8.8 (4 H, CH_2).

Compound purity was determined by glpc on a 2 m × 0.25 in. column of DC 200 silicone oil at 173° and a helium pressure of 15 psi in a Perkin-Elmer Model 154D instrument. When a 15-mμ sample (13% chloroform solution) was injected, a single peak was found having a retention time of 14.5 min.

1,7,7-Trimethyl-2-azabicyclo[2.2.1]heptan-3-one (IV) was prepared from aminodihydrocamphonanic acid following the procedure of Noyes and Potter.[25] The aminodihydrocamphonanic acid precursor was synthesized from camphoric anhydride as described by Noyes.[24] Purification of the lactam was accomplished by sublimation at 105° (0.5 mm); mp 187–188° (lit.[25] mp 188–189°); $[\alpha]^{25}\text{D}$ 70.1° (0.0965 g/cc, ethanol) [lit.[25] $[\alpha]^{30}\text{D}$ 72.8° (0.1 g/cc, petroleum ether (bp 30–60°))].

The nmr spectrum ($CDCl_3$) consisted of a broad band at τ 3.42 (1 H, NH), three singlets at 8.83, 8.97, and 9.12 (9 H, CH_3), an unresolved singlet at 7.77 (1 H, CH), and a number of complex multiplets at 7.8–8.6 (4 H, CH_2).

An analysis by gas chromatography of an 18-mμ sample (12% chloroform solution) showed a single peak having a retention time of 15.3 min.

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Protonation of Peptides. I. The Behavior of a Model Diamide and of Poly-γ-ethyl-L-glutamate in Strong Acid–Water Mixtures

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Contribution from the Institute of Organic Chemistry, University of Padua, Padua, Italy. *Received August 5, 1968*

Abstract: Poly-γ-ethyl-L-glutamate dissolves in concentrated sulfuric acid and in methanesulfonic acid. Circular dichroism measurements showed that it was in the coil form in both solvents. The addition of water produced the α-helical form of the polymer. At room temperature the transition took place in 57–62% sulfuric acid by weight, and in 75.5–78% methanesulfonic acid. The diamide N-benzoylglycyl-*n*-propylamide was examined spectrophotometrically in aqueous mixtures of the two acids in order to determine the region in which the second protonation occurred. Assuming that the glycylamide group was protonated first, the benzamide portion was half-protonated in 74% sulfuric acid. Protonation was not complete in 99.0% methanesulfonic acid, and it was tentatively concluded that the second protonation was much less than half-completed in 75% acid. From these results it was hypothesized that in sulfuric acid–water mixtures no more than 50% of the amide groups of the polymer helical residues were protonated early in the transition, and that the percentage might be much less in methanesulfonic acid–water solutions in which the transition started. The cooperative character of the helix–coil transition was evident from the relatively small change in acidity which was required to produce it, in contrast to the range of acidity which is required for extensive amide protonation.

The connection between the helix–coil transition of polyamino acid derivatives and the protonation of the amide groups of these compounds is not clear at the present time. Klotz and his coworkers have maintained that in trifluoroacetic acid–chlorinated hydrocarbon mixtures there is an initial protonation of amide groups at low acid concentration without disruption of the helix, followed (at much higher acid concentration) by an additional reaction between the trifluoroacetate–amide ion pairs and trifluoroacetic acid. The latter reaction, which forms homoconjugate ion pairs, is presumably responsible for the disruption of the helix. From the examination of simple models and of polymers they have shown some evidence for this thesis by near-infrared spectroscopic studies, and density, nmr, and conductivity measurements.[2-4] Stewart, *et al.*, compared the nmr spectra of poly-L-alanine and poly-DL-alanine[5] with those of the simple models N-methylacetamide and N,N-dimethylacetamide[6] in trifluoroacetic acid–chloroform mixtures. They concluded that whereas there is

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