Raman Spectroscopy of Proline Oligomers and Poly-L-proline

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Abstract: The Raman spectrum of helical poly-L-proline with cis peptide bonds (form I) has been obtained. Comparison of this spectrum with the previously reported Raman spectrum of poly-L-proline II reveals a number of lines which are sensitive to the conformation; some of these correpond to bands seen in the infrared spectra—others, however, appear to be unique to the Raman spectra. In addition, it is noted that the relative intensities of certain lines are sensitive to conformation. The spectrum obtained from the "collapsed" form of poly-L-proline precipitated from concentrated calcium chloride solution is also presented. Finally, Raman spectra are presented for three proline oligomers (trimer, tetramer, and pentamer) with the *tert*-amyloxycarbonyl blocking group on the terminal imino nitrogen. All three oligomers appear to have the poly-L-proline II stucture in aqueous solution; in addition, the three solids recovered from these aqueous solutions by lyophilization possess this helical structure. On the other hand a DP > 4 is necessary for the solids recovered in the absence of water, or dried in a vacuum oven, to assume this left-handed helical structure.

Poly-L-proline exists in at least two distinct conformational forms whose structures have been elucidated by X-ray crystallography.[1,2] These forms correspond to those predicted from vacuum calculations, using steric and electrostatic interactions and minimizing energy.[3,4] However, the form obtained depends upon the solvent from which the polymer is precipitated. The nomenclature used to distinguish the two forms was introduced by Blout and Fasman,[5] and was based on the differences observed in the infrared spectra of the solid. Precipitation of the polypeptide directly from the polymerization mixture by ethyl ether gives form I; on the other hand if form I is permitted to stand in glacial acetic acid for several days, form II can be precipitated with ether. Form I corresponds to a right-handed helix with cis peptide bonds, while form II is a left-handed helix with the peptide bonds in the trans configuration. Steinberg, *et al.*,[6] have followed the reversible transformation I $\rightleftarrows$ II, induced by solvent change, using infrared spectroscopy and the change from $[\alpha]^{25}D$ $+40°$, characteristic of form I, to $[\alpha]^{25}D$ $-540°$ indicative of form II.

Isemura, *et al.*,[7,8] have reported the infrared spectra, ultraviolet absorption spectra, optical rotatory dispersion spectra, and circular dichroic spectra for a series of L-proline oligomers synthesized in their laboratory. From these data they conclude that the oligomers in the solid state with DP > 4 have a helical structure with a left-handed threefold screw axis identical with poly-L-proline II. In addition, their solution studies indicate that the tetramer also assumes the poly-L-proline II structure in solution.

It is apparent from the foregoing discussion that infrared spectroscopy is useful in distinguishing between the two helical conformations of poly-L-proline. On the other hand, Raman spectroscopy complements the data available from infrared spectroscopy and its application to biopolymers should be worthwhile. Edsall, *et al.*,[9,10] have tabulated Raman spectra for many amino acids and a few polypeptides, but application of Raman spectroscopy to conformational studies has been limited due to experimental difficulties. More recently Smith, Walton, and Koenig[11] obtained the laser-excited Raman spectrum of poly-L-proline II in aqueous solution and the solid state. Their results support the concept of a similar conformation for the two environments. There are, however, advantages in obtaining the Raman spectrum for the second conformation, since some unique Raman active lines which are conformationally sensitive may be seen. The broader frequency range of Raman spectroscopy allows the low-energy skeletal vibrations to be observed, and these should prove to be sensitive to conformation.

A number of biopolymers have been reported to undergo structural change in the solid state, due to the presence or absence of water vapor. Elliott, *et al.*,[12] and Blout and Lenormant[13] have shown that control of humidity can induce a helix β transition in the solid state for poly-L-lysine with low humidity favoring the β form; Lenormant, *et al.*,[14] have observed a similar effect for poly-L-glutamic acid. Recently de Lozé and Josien[15] reported a hydration-induced transition from poly-L-proline I to poly-L-proline II. However, in these studies both the observed conformations were available to the dried polymers and the water merely caused a change from one stable state to the other. Also, the use of infrared spectroscopy for these observations is not ideal since the absorption of

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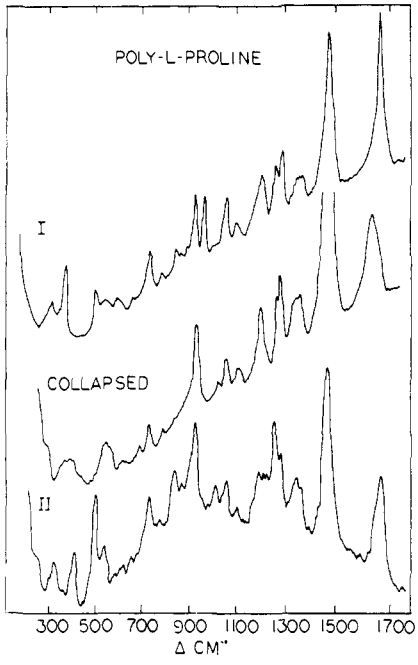

Figure 1. Raman spectra of poly-L-proline: form I, collapsed, and form II, in the solid state.

water itself may make interpretation difficult. In the present study, the presence of water induces the tripeptide to assume a helical form which does not appear to be found in the "dry" state. In addition, the use of Raman spectroscopy eliminates the problem of interpretation because of the very low scattering of water and thus the spectral changes can be attributed to structural changes and/or the effect of hydrogen bonding on the group vibrations. Since water plays a fundamental role in the structure of biological macromolecules, even in the solid state, it is expected that Raman spectroscopy may play an increasingly important part in the elucidation of molecular structure.

This paper presents the laser-excited Raman spectra for poly-L-proline I, preliminary data for a collapsed form, and the Raman spectra for three proline oligomers. The work with the oligomers complements the infrared spectra already published.[7,8]

Experimental Section

Poly-L-proline was obtained from Mann Research Laboratories, Batch No. U2361. A 5% solution in glacial acetic acid was diluted with 10 vol of *n*-propyl alcohol; the resulting solution was left at room temperature for 4 days to convert the polymer to form I. The poly-L-proline I was then precipitated with ethyl ether. The precipitated solid was dissolved in *n*-propyl alcohol and reprecipitated with ethyl ether. This procedure was repeated several times until a clean dry powder, free of acetic acid, was obtained. The resulting polymer was judged to be poly-L-proline I by the presence of infrared absorption bands at 1355 and 960 cm^{-1} (KBr disks). Form II was thought to be absent since no infrared band appeared at 400 cm^{-1}.

Recent attention has focused on a so-called "collapsed" form of poly-L-proline which is produced in concentrated calcium chloride solutions.[16,17] Although we have been unable to obtain convincing Raman spectra of the solution form, material precipitated with ether from 6 *M* calcium chloride solution yields a unique spectrum. This material may be peculiar to the preparation method; however, we are inclined to identify it as the collapsed form.

Three proline oligomers were obtained from Dr. Sakakibara, Institute for Protein Research, Osaka University, *viz.*, *tert*-amyloxycarbonyl-L-prolylprolylproline (trimer), *tert*-amyloxycarbonyl-L-prolylprolylprolylproline (tetramer), and *tert*-amyloxycarbonyl-L-prolylprolylprolylprolylproline (pentamer). Raman spectra were measured on the solids as supplied, aqueous solutions of the oligomers (deuterium oxide was used for the carbonyl stretching region since water scattering made assignment of the frequencies difficult), and the solids recovered from the aqueous solutions by freeze-drying.

The Raman spectra were obtained using an instrument built in this department. The excitation source is an argon ion laser having an output power of approximately 200 mW at 4880 Å. The beam passes through a narrow band pass filter and is reflected upward and focused onto the sample. Light scattered by the sample is collected by a lens and focussed onto the entrance slit of a Spex 1400 double monochromator. The output of the monochromator is detected by an ITT FW130 startracker phototube which is cooled to −20° by a thermoelectric cooler. The photon pulses from the phototube are counted, passed through a discriminator, and displayed on a strip chart recorder.

The solid samples were pressed into a holder designed to place the smooth compacted surface at 20° to the 4880-Å laser beam and the scattered radiation was sampled to 90° to the incident laser beam. Saturated aqueous solutions were illuminated in glass vials with approximately 1-cm^{-1} path lengths and the scattered radiation was again collected at 90° to the main beam. The instrument was periodically calibrated with the Raman spectrum of benzene and at the completion of each run, the wavelength marker was checked against the 5460-Å mercury line. The reported frequencies are accurate to ±4 cm^{-1}.

Results and Discussion

A. Poly-L-proline I. The Raman spectra obtained for poly-L-proline form I and the collapsed form, along with the spectra previously reported, for poly-L-proline II, are shown in Figure 1. Comparison of these spectra with the spectrum of L-proline shown in Figure 2 reveals several bands which are common to the monomer and the various forms of the polymer. These common bands could possibly arise from vibrations present in the L-proline repeating unit and are shown in Table I. Tentative assignments, based on the data published for pyrrolidine[18] and 2-pyrrolidone,[19] are also shown in Table I for a number of these common bands.

Table I. Tentative Assignments of the Raman Lines Common to the Monomer and Both Polymeric Forms of L-Proline

Proline, cm^{-1}	Poly-L-proline I, cm^{-1}	Poly-L-proline II, cm^{-1}	Lit.,[a] cm^{-1}	Tentative assignment
1446	1446	1447	1454[b]	CH_2 bend
1261	1261	1266	1287[b]	
1237	1237	1241	1221[b]	
		1198		
	1187	1188		CH_2 wagging–twisting
1172		1176	1161[c]	
1080	1080	1093	1082[b]	CH_2 rocking
1055	1044	1046	1025[b]	CH_2 wagging
983	1000	957	980[b]	Ring stretching
914	917	919		Ring breathing
896			902[b]	
840	838	839	838[d]	CH_2 out plane bend

[a] Values reported for pyrrolidine, 2-pyrrolidone, and pyrrole. [b] Reference 18. [c] Reference 19. [d] A. R. Kalvetsky, *Quart. Rev., Chem. Soc.*, **4**, 353 (1959).

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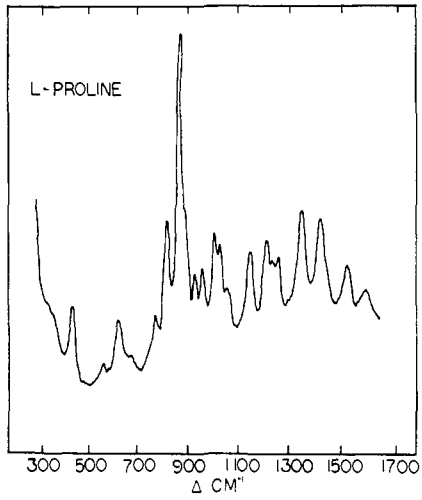

Figure 2. Raman spectrum of L-proline, in the solid state.

Table II summarizes the major differences found in the Raman spectra of the two forms. In addition, the differentiating features are shown for infrared spectroscopy. From this summary it is evident that Raman spectroscopy can be useful in distinguishing the conformations available to polypeptide chains.

Table II. Summary of the Raman Scattering Lines and Infrared Absorption Bands Which May Be Used to Distinguish the Two Forms of Poly-L-Proline

Raman		Infrared	
Poly-L-proline I, cm^{-1}	Poly-L-proline II, cm^{-1}	Poly-L-proline I, cm^{-1}	Poly-L-proline II, cm^{-1}
		1355	
	1198		
1187	1187		
	1176		
957	1000	960	
781			
	722		
662			670
540	530		
363	400		400

The 1647-cm^{-1} line in the spectrum of poly-L-proline I is assigned to the carbonyl stretching mode. This frequency is experimentally equivalent to the 1650-cm^{-1} line assigned to this vibration in poly-L-proline II. However, if the intensities of these carbonyl stretching modes are compared with the intensities of their respective CH_2 bending modes at 1446 cm^{-1}, it is seen that the intensities for the bending mode and the carbonyl mode are approximately equal for form I, whereas for form II the carbonyl scattering is only one-half that of the CH_2 bending mode. By contrast, the available infrared data do not indicate this intensity difference and this difference could reflect the sensitivity of the Raman technique to the symmetric A modes rather than the E modes which dominate in the infrared. Band intensities are known to be first-order perturbation effects, whereas band frequencies are second-order perturbation effects; thus, the change in relative scattering could reflect the effect of the slightly altered carbonyl environment due to the different conformations.

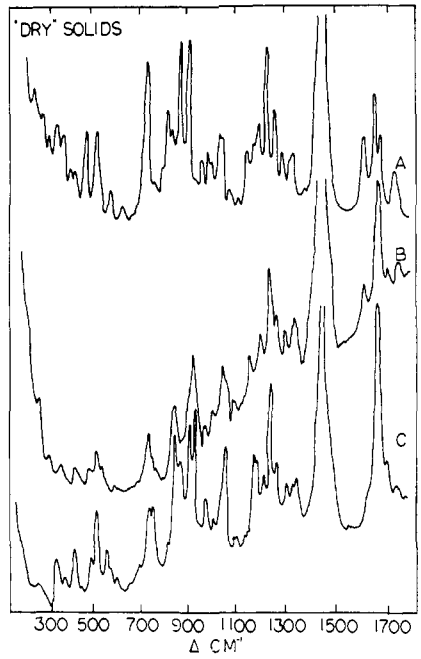

Figure 3. Raman spectra of *tert*-amyloxycarbonylprolyprolyl-proline (A), *tert*-amyloxycarbonylprolylprolylprolylproline (B), and *tert*-amyloxycarbonylprolylprolylprolylprolylproline (C), as dried solids.

On the other hand, it is possible that the sample of poly-L-proline in form II had a small amount of water bound weakly to the carbonyl groups even in the solid state. This could then account for the observed difference in band intensity without the second-order perturbation in band frequency. Examination of the spectrum for the dry tetramer, shown in Figure 3B, reveals that this oligomer has many of the poly-L-proline II features, including a reduced carbonyl intensity. In addition, comparison between the "dry" solids, shown in Figure 3, and the wet solids, shown in Figure 4, indicates that the incorporation of water gives rise to a broadening of the 1180-cm^{-1} peak—a trend that is even more obvious if the aqueous solution spectra are observed; however, the poly-L-proline II spectrum in Figure 1 does not have this broadening. Thus, it is unlikely that water is bound to the polypeptide and the difference in the relative intensities mentioned above is due to the different conformations.

The overlapping group of bands peaking at 1315 and 1337 cm^{-1} are common to both polymeric forms, but absent in the monomer. These bands will be discussed in more detail when the oligomers are treated since the development of this complex is more obvious in the oligomers. Another spectral difference between the two polymeric forms appears in the region of the CH_2 twisting and wagging modes. Both the polymers and the monomer have lines at 1239, 2, and 1264 ± 3 cm^{-1}; however, comparison of the relative intensities of these two lines reveals a similar pattern for poly-L-proline II and the monomer which differs from the form I pattern (where the 1266-cm^{-1} line dominates the 1237-cm^{-1} line). Another variation in the Raman spectra of the two forms is found in this same region, for poly-L-proline II has three lines at 1176, 1190, and 1198 cm^{-1} of equal intensity, whereas poly-L-proline I has a single line at 1187 cm^{-1} and L-proline has a single line at 1172 cm^{-1}.

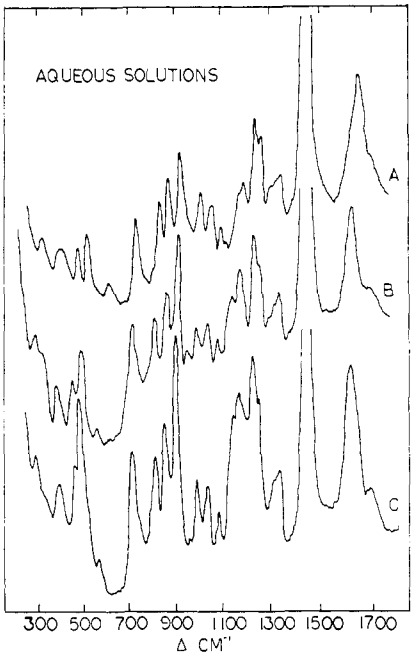

Figure 4. Raman spectra of aqueous solutions of *tert*-amyloxyprolylprolylproline (A), *tert*-amyloxycarbonylprolylprolylprolylproline (B), and *tert*-amyloxycarbonylprolylprolylprolylprolylproline (C).

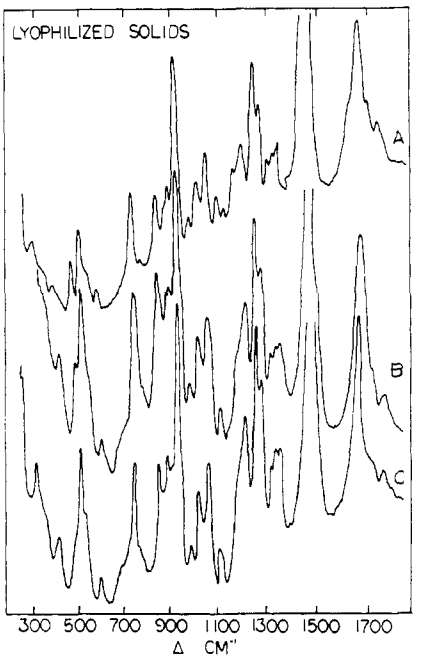

Figure 5. Raman spectra of lyophilized *tert*-amyloxycarbonylprolylprolylproline (A), *tert*-amyloxycarbonylprolylprolylprolylproline (B), and *tert*-amyloxycarbonylprolylprolylprolylprolylproline (C).

An important feature of the infrared spectrum of poly-L-proline I is the rather strong band at 960 cm^{-1}, a band which is absent in form II. The Raman spectra also show this difference. Form I is seen to have a strong line at 957 cm^{-1} which can be identified with the infrared band at 960 cm^{-1}. The Raman line at 1000 cm^{-1} appears to be unique to form II and corresponds in relative intensity to the 1002-cm^{-1} band seen in the infrared spectrum.

Two weak Raman lines, which are unique to form I, are found at 781 and 662 cm^{-1}. There is a line at 730 cm^{-1} which is common to both forms, and could be assigned to a CH_2 rocking mode. Finally, a line of medium strength is seen at 363 cm^{-1} which can serve to identify form I by Raman spectroscopy, just as the 400-cm^{-1} band has come to be associated with form II in infrared spectroscopy. Both these bands are most likely due to skeletal vibrations; however, the 363-cm^{-1} line of form I is not seen in the infrared, whereas the 400-cm^{-1} peak of form II is common to both the Raman and infrared spectra.

B. Proline Oligomers. The spectra obtained from the oligomers, as supplied in the solid state and aqueous solution, respectively, are shown in Figures 3 and 4. The spectra for the solids which were recovered from these aqueous solutions by lyophilization are shown in Figure 5.

Comparison of the spectra shown in Figure 5 with the corresponding ones in Figure 3 reveals a number of differences. There is a broadening of the 1650-cm^{-1} peak which results in rather poor resolution of the multiple peaks of the original solids. Likewise, the peak at 1180 cm^{-1} is broadened in a similar manner.

There is a broadening of both these peaks in the spectra shown in Figure 4 obtained from the aqueous solution. Finally, the distinctions between the various spectra obtained from the oligomers as supplied are partially lost when the spectra of the aqueous solutions, or lyophilized oligomers, are compared. Under these conditions, the spectra from all three oligomers are seen to have features common to poly-L-proline form II. In particular, the lines at 400 and 1000 cm^{-1}, unique to form II, are seen in all three oligomers. Each of the differences mentioned above can be explained if "water of crystallization" is present in the lyophilized solids. Then the broadening of the carbonyl scattering peak would follow from hydrogen bonding and the same hydrogen bonding of solvent would result in stabilization of the otherwise random trimer into a geometry similar to that of polyproline II. In further support of this suggestion, it is observed that extensive drying of the solids of both the trimer and tetramer in a vacuum oven after lyophilization results in spectra similar to those shown in Figure 3.

The above explanation suffices for the minor differences mentioned. However, a few additional features evident in the spectra of the pentamer (C) require interpretation. In particular, there are weak peaks at 360, 542, and 960 cm^{-1} in the original spectra of the pentamer (Figure 3C). In the previous section, these peaks were shown to be indicative of form I. These bands were absent from the solution spectrum (Figure 4C), and did not return with extensive drying of the lyophilized solid. In this connection it is interesting to note that Rothe, *et al.*,[20] report that helical conformation begins with triproline in solution and their studies indicated that both forms I and II were possible, depending on the solvent. However, starting with the hexapeptide, they could isolate form I in the solid, whereas the solid tetramer was found to take up form II conformations. Upon dissolution of the pentamer

(20) M. Rothe, R. Theysohn, K. D. Steffen, H. J. Schneider, M. Zamani, and M. Kostrzewa, *Angew. Chem., Int. Ed. Engl.*, in press.

in water, a small fraction of the solid was insoluble in water, in contrast with the tetramer and form II polymer which were both quite soluble in water. Thus, it is probable that the pentamer as originally supplied had both forms I and II present, the latter predominating. This proposition is supported by solubility, Raman scattering lines at 360, 540, and 960 cm^{-1}, and finally the strong scattering of the carbonyl band.

In the discussion of the spectra obtained from the polymers, the assignment of the lines at 1315, and 1337 cm^{-1} was deferred. If the spectra of the three oligomers in Figure 5 are compared with the spectrum of L-proline in Figure 2, it is seen that the monomer has a line occurring at 1294 cm^{-1}, the trimer at 1288 cm^{-1}, the pentamer and the polymer both at 1307 cm^{-1}. Since pyrrolidine has a CH_2 twisting mode at 1287 cm^{-1},[13] the line at 1315 cm^{-1} for the polymer is assigned to a CH_2 twisting mode. The assignment of the 1347-cm^{-1} line is less clear; however, the monomer has a line at 1369 cm^{-1} which may be identified with the CN-stretching mode seen at 1424 cm^{-1} for pyrrolidone and 1369 cm^{-1} for *N*-bromopyrrolidone. Based on this assignment, the 1337-cm^{-1} line of the polymer may be seen to arise from the C–N stretching mode.

A feature of the spectra of the two forms of the polymer was the variation in the ratio of the scattering at 1650 cm^{-1} to the scattering at 1446 cm^{-1}. All three oligomers have a similar ratio to that found for form II. Likewise, the intensities of the scatterings at 1266 and 1240 cm^{-1} are indicative of form II rather than form I. The multiple lines in the carbonyl region may be assigned to ester carbonyls, internal carbonyls, and free carboxyl groups as in the infrared;[7] however, the change in intensities is interpreted as being due to a simple increase in the number of internal carbonyls over end group carbonyls as the chain lengthens. The 1650-cm^{-1} line is interpreted as the vibration of an imino acid peptide-linked carbonyl; however, the persistence of a weak band at 1686 cm^{-1} is confusing.

Finally, it is worth noting a couple of additional changes in the spectra as the oligomer is progressively lengthened from 3 to 5 units. A line at ~476 cm^{-1} is seen to be diminished in intensity and since this line is absent both from L-proline and poly-L-proline I or II, it is assigned to a skeletal mode of the *tert*-amyloxycarbonyl group blocking the terminal nitrogen of these oligomers.

If the spectra in Figure 5 are compared, it is evident that the trimer has a weak line at ~880 cm^{-1} whereas the tetramer has two lines in this region, one at ~880 cm^{-1} and the other at 871 cm^{-1}. Finally, the pentamer has only one line at 869 cm^{-1} with a slight shoulder at 880 cm^{-1}. For comparison, form II of the polymer has a line at 869 cm^{-1} and is similar to the pentamer and since there are no corresponding bands in the spectra of the monomer, they are likely to be skeletal vibrations.

Conclusion

The Raman spectrum of poly-L-proline I is seen to have a line at 957 cm^{-1} which corresponds to the 960-cm^{-1} band in the infrared spectrum. In addition, lines at 781, 662, and 363 cm^{-1} are unique to the Raman spectrum and sensitive to conformation. Finally, comparison of the spectra obtained for form I and form II shows a reduction in the carbonyl scattering at 1650 cm^{-1} for the latter and a reversal of the intensities of the lines at 1264 and 1239 cm^{-1}.

From the Raman spectra of a series of proline oligomers, it is concluded that in aqueous solution and lyophilized solids, the oligomers from the trimer upward are capable of existing in a helical form similar to poly-L-proline II. However, the spectra from dried solids indicate that the tetramer is the first to have a stable helical form and this is in agreement with the conformational calculations done on these oligomers. The failure to obtain any oligomers in the cis form, as solids, is also in agreement with these calculations. Recent solution studies[20] indicate, however, that the cis structure may be possible for DP $\geq$ 3 if no imino blocking group is present.

Finally, this work has emphasized the usefulness of comparing band intensities as well as the more commonly treated band frequencies, since it has been shown that the relative intensities of the carbonyl stretching modes and the various CH modes are sensitive to the conformation of the polypeptide chain.

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Communications to the Editor

π-Complexed β-Arylalkyl Derivatives. III. The Acetolysis of Some Chromium Tricarbonyl Complexed 2-Benzonorbornenyl Methanesulfonates[1]

Sir:

The acetolyses of chromium tricarbonyl complexed neophyl-type methanesulfonates are enhanced and yield π-arylchromium tricarbonyl migrated products.[2] In order that we might infer whether the π-bonded metal moiety prefers to precede or to follow[3] the migrating aryl to which it is bonded in these acylic derivatives, we have examined the relative acetolysis rates and products of the isomeric *endo*- and *exo*-chromium tricarbonyl

(1) Portions of this work were presented at the 159th National Meeting of the American Chemical Society, Houston, Tex., Feb 1970, Abstract ORGN 133.

(2) R. S. Bly, R. C. Strickland, R. T. Swindell, and R. L. Veazey, *J. Amer. Chem. Soc.*, **92**, 3722 (1970).

(3) We use the terms "precede" and "follow" in the geometric sense only and do not intend them to imply anything about the electronic nature of the process.