

Poly(*trans*-5-methylproline)의 변광회전에 따른 용액의 성질변화

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Changes in Solution Properties of Poly(*trans*-5-methylproline) During Mutarotation

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요 약. Poly(*trans*-5-methylproline) (PTMP)의 변광회전에 따른 CD, ORD 및 uv 스펙트럼의 변화를 관찰하였다. PTMP의 정방향 변광회전은 유기강산이나 trifluoroethanol (TFE) 속에서 일어나며 역방향 변광회전은 TFE용액을 과량의 지방족 알코올로서 희석시킬때 일어났다. 정방향과 역방향 변광회전시의 CD, ORD, 및 uv 스펙트럼의 변화는 polyproline 변광회전시의 변화와 평형하게 일어났다. PTMP의 정방향 변광회전시 α CH- 양성자의 화학적 이동은 0.3 ppm downfield로 이동하였고 환원 점도는 0.15에서 0.26(dl/g)로 증가하였다 적당한 용매의 혼합물에서는 형태 I 과 II는 평형을 이루었다. 이러한 모든 용액성질의 변화는 polyproline의 변화와 유사하기 때문에 I, II의 두 형태는 polyproline 형태 I, II와 같을 것이며 형태 I은 모두 시스아미드 결합을 가진 helix 이고, 형태 II는 모두 트랜스 아미드 결합을 가진 helix일 것이다.

ABSTRACT. Changes in CD, ORD and uv-spectra during the mutarotation of poly(*trans*-5-methyl-L-proline) (PTMP) were studied. The forward mutarotation of PTMP occurred in strong organic acids and trifluoroethanol, while the reverse mutarotation was observed by dilution of the trifluoroethanol solution with excess aliphatic alcohols. The changes in CD, ORD and uv-spectra during the forward and reverse mutarotation proceeded parallel to those found for the mutarotation of polyproline. The chemical shift of the α CH-proton was shifted downfield about 0.3 ppm during the forward mutarotation. The reduced viscosity for the forward mutarotation increased from 0.15 to 0.26 (dl/g) during 5 days. The equilibrium between form I and form II was established in an appropriate solvent mixture. All changes in solution properties mentioned above are similar to those found for polyproline. These results support that the two forms of PTMP are the same conformations as polyproline form I and form II, *i. e.*, a right-handed helix with all *cis* amide bonds and a lefthanded helix with all *trans* amide bonds.

INTRODUCTION

In the previous paper¹ we have reported the

synthesis and conformational analysis of optically active poly(*trans*-5-methylproline) (PTMP). It was found that PTMP exists in two confor-

mations in the solid state as well as in solution in appropriate solvents, and the two forms can be interconverted by changing the solvent composition. Based on the similarities on ORD, CD, uv and ir-spectral data between polyproline (PP) and PTMP, it was proposed that the two conformations of PTMP are the same conformations as PP forms I and II, *i.e.*, a right-handed helix with all *cis* amide bonds and a left-handed helix with all *trans* amide bonds.

In the case of PP², it was found that the forward and reverse mutarotations resulted in changes of various solution properties of PP such as optical properties and viscosity, which were characteristic of *cis-trans* isomerization of amide bonds. If the two forms of PTMP are the same conformations of PP, as proposed, we can expect the similar changes in solution properties during the mutarotation to those observed in PP. For this purpose, an investigation of the solution properties of PTMP during the mutarotation was carried out. This paper reports the changes in CD, ORD, uv, nmr and ir-spectra and the changes in viscosity during the mutarotation of PTMP. Furthermore, the conformation of PTMP in acidic solution and the establishment of an equilibrium state in a binary solvent mixture are also reported.

EXPERIMENTAL

Polymerization. PTMP was synthesized by bulk polymerization of *trans*-5-methylproline-N-carboxyanhydride with sodium methoxide, as described previously¹. The following three polymer were used in this investigation (Table 1).

Infrared Spectra. ir-Spectra were taken with a Perkin-Elmer 257 grating Infrared Spectrophotometer.

Optical Measurements. CD, ORD and uv-measurement were performed with a Jasco ORD CD uv-5 Spectropolarimeter at ambient temper-

Table 1. PTMPs and their physical data.

polymer No.	I/A ^a	Mr ^b	[η] ^c
1	5	600	0.18
2	10	82 ^a	0.15
3	100	—	0.26

^a Initiator to anhydride ratio.

^b Measured by VPO in trifluoroethanol.

^c In trifluoroethanol at 25 °C.

ature (25°C). A 0.1 mm cell was used and polymer concentrations were approximately 1 mg/ml.

Viscosity Measurements. Viscosity measurements were made at 25 ± 0.02°C with Cannon-Ubbelohde viscometer.

nmr-Spectra. nmr-spectra were recorded with Varian T-60.

RESULTS

Changes in CD, ORD and uv-Spectra During Mutarotation. Two forms of PTMP (form I and II) showed different uv, CD and ORD-spectra in appropriated solvents¹. Form II is stable in strong organic acids such as methanesulfonic, trifluoroacetic and dichloroacetic acid, and also in trifluoroethanol (TFE). Form I is stable in the solvent mixtures TFE-*n*-butanol (*n*-BuOH) and TFE-*n*-propanol (*n*-PrOH), in which the alcohol component is in excess. The two forms are interconvertible by changing the solvents. When form I was dissolved in a strong organic acid, the forward mutarotation (form I → form II) occurred, but was too fast to be followed with optical measurements. The forward mutarotation in TFE is slow, being completed in 5 days.

The reverse mutarotation of PTMP (form II → form I) occurred upon dilution of the TFE solution with excess aliphatic alcohols such as *n*-BuOH and *n*-PrOH. Neither form I nor form II of PTMP was soluble in aliphatic alcohols.

However, when a TFE solution of PTMP was diluted with aliphatic (*n*-BuOH and *n*-PrOH) to the TFE-alcohol volume ratio of 1:4, no precipitation was observed after several months. The reverse mutarotation of PTMP in TFE-*n*-BuOH (1:4 v/v) was completed in 7 days. The directions of the mutarotations of PP and PTMP were found to be same in a similar solvent.

The time-dependent ORD, CD and uv-spectra were recorded during the forward and reverse mutarotations. Fig. 1 shows the CD-spectra during the forward mutarotation of PTMP in TFE at room temperature (25 °C). PTMP form I, when measured in TFE-*n*-BuOH in which form I is stable (Fig. 4), gave three CD bands—a positive at 216 m μ and a negative band at 202 m μ , which are generally accepted as $\pi \rightarrow \pi^*$ bands, and a negative band at 234 m μ . When the CD-spectrum was recorded within 8 minutes

after dissolving in TFE (Fig. 1), the weak negative band at 234 m μ had already disappeared. During the forward mutarotation, the positive band at 216 m μ characteristic of form I was red-shifted and its intensity was decreased. Hence, a small positive lobe at 224 m μ characteristic of form II remained after completion of the mutarotation. The increase in intensity and the red-shift of the negative band at 202 m μ were observed during the forward mutarotation. The final CD-spectrum is identical to the CD-spectrum of form II in the same solvent.

The change in the ORD-spectra of PTMP during the forward mutarotation is shown in Fig. 2. Immediately after dissolving form I, a positive band at 224 m μ and a negative band at 207 m μ appear. These bands are similar to the ORD-spectrum of form I in TFE-*n*-BuOH (Fig. 5). In the course of time the positive band disappeared, and a red-shift and a decrease intensity of the negative band were

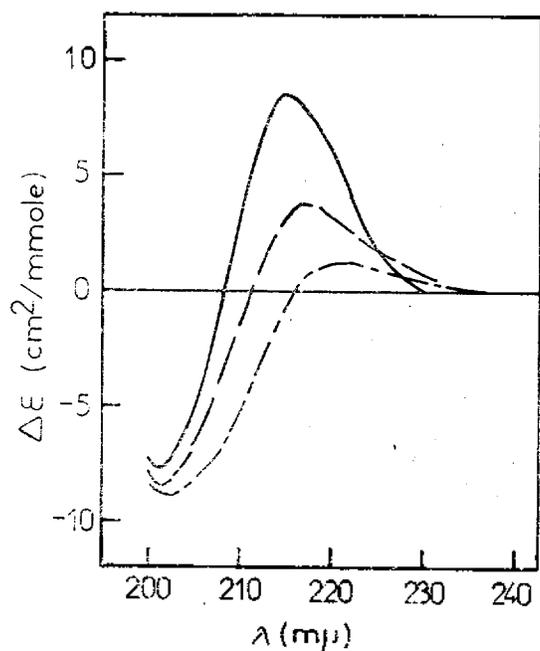


Fig. 1. Changes in CD-spectra of PTMP during the forward mutarotation in TFE at room temperature; After dissolving (—), after 1 day (---) and after 5 days (- - -).

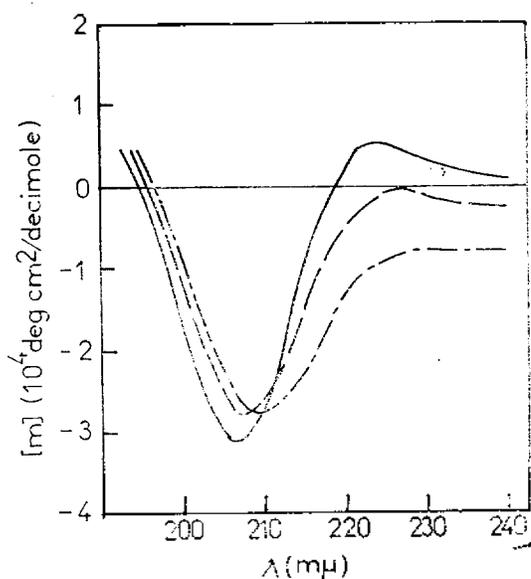


Fig. 2. Changes in ORD-spectra of PTMP during the forward mutarotation in TFE at room temperature; after dissolving(—), after 1 day (---) and after 5 days (- - -).

observed, so that the ORD-spectrum of form II resulted after 7 days.

It is interesting to compare the changes in CD and ORD-spectra during the forward mutarotation of PTMP with the corresponding spectra of PP in the same solvent reported by Bovey and Hood.^{3,4} The changes in intensity and the shifts of the CD and ORD bands during the forward mutarotation are parallel in both polymers. This result could be an evidence for that the two forms of PTMP are the same conformations of pp form I and II.

The absorption band of PTMP form I in TFE occurred at 209 $m\mu$ with higher intensity ($\epsilon=8,200$), whereas form II gave a absorption band at 203 $m\mu$ with lower intensity ($\epsilon=6,700$)¹. Consequently the changes in wave length of band maxima and extinction coefficients during the mutarotation were expected. Fig. 3 shows the changes in wave length of absorption maxima and extinction coefficients during the forward mutarotation of PTMP at room temperature (25°C). Initially the changes are very fast, and after 5 days no further changes are observed, *i.e.*, the mutarotation was completed in this interval.

The CD and OR-spectra of PTMP for the reverse mutarotation were shown in Fig. 4 and 5. The CD and ORD-spectra of form I after completion of reverse mutarotation are somewhat different from those observed at the beginning of the forward mutarotation; a small negative lobe at 234 $m\mu$ characteristic of form I was found in the CD-spectrum after completion of the reverse mutarotation. However, this lobe could not be recorded at the beginning of the forward mutarotation (Fig. 1). The intensity of the peak at 224 $m\mu$ ($[\eta]=12,500$) in the ORD-spectrum of PTMP form I after the reverse mutarotation (Fig. 5) was found to be much higher than that ($[\eta]=7,000$) at the beg-

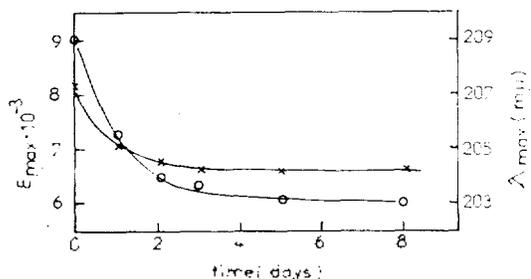


Fig. 3. Changes in uv extinction coefficients (x) and absorption maxima (o) of PTMP during the forward mutarotation in TFE at room temperature.

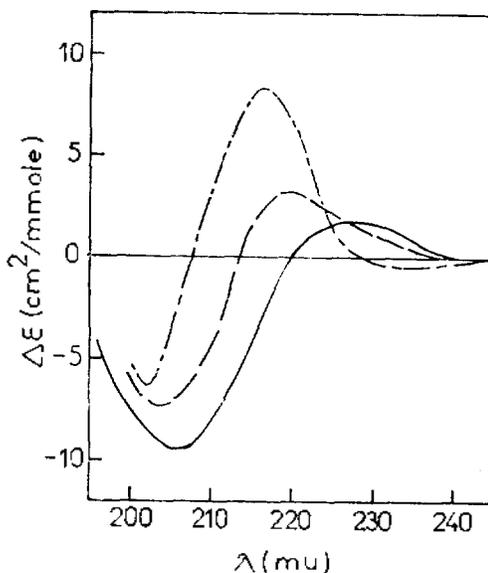


Fig. 4. Changes in CD-spectra of PTMP during the reverse mutarotation in TFE-*n*-BuOH (1:4 v/v) at room temperature: after dissolving (—), after 1 day (---) and after 40 days (- - -).

inning of the forward mutarotation (Fig. 2). These differences seem to be due to the rapid decrease in intensity of both bands at the beginning of the forward mutarotation in TFE.

Nmr-Spectra of Form I and Form II. The nmr-spectra of two forms of PP were studied by many authors⁵⁻⁷, who found that the α CH-protons in form I and II showed different chemical shifts, form I at $\delta=4.5$ ppm and form

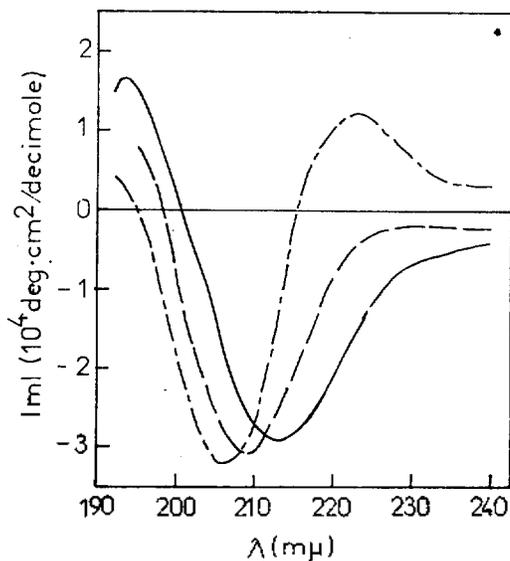


Fig. 5. Changes in ORD-spectra of PTMP during the reverse mutarotation in TFE-*n*-BuOH (1:4 v/v) at room temperature: after dissolving (—), after 1 day (---) and after 40 days(- · -).

II at $\delta=4.8$ ppm. The conformational change from I to form II resulted in 0.3 ppm downfield shift. The other protons in the pyrrolyl ring had nearly the same chemical shifts in both forms. In the studies of proline oligomers, Okayabashi *et al.*⁵ found that the resonance shielding value of the α CH-protons in the terminal residues of form II was shifted somewhat upfield in comparison with the value of the internal residues, so that a broad peak appeared for the α CH-protons in the nmr-spectrum of proline oligomers.

PTMP form I was dissolved in TFE- d_3 , in which the forward mutarotation occurred, and the nmr-spectra recorded after dissolving and after 2 weeks are shown in Fig. 6. The CH_3 and β and γ CH₂-protons gave peaks at $\delta=1.35$ and 2.35 ppm, respectively, which depend little on their conformations. The α and δ CH-protons of form I gave a broad peak at 4.0 with a shoulder at 4.3 ppm. This signal became wider

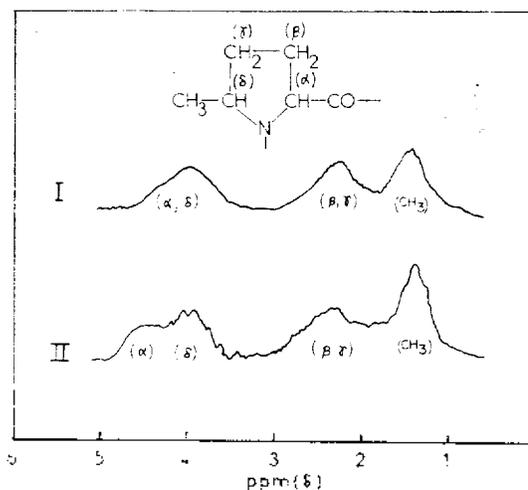


Fig. 6. Nmr-Spectra of PTMP form I and form II in TFE- d_3 .

during the period of mutarotation and eventually formed two broad peaks at 4.5 and 4.0 ppm after the completion of the forward mutarotation. Since the chemical shift of the δ CH₂-protons in PP was not changed, and since the signal of the α CH-protons was shifted downfield during the forward mutarotation of PP, it is reasonable to assign the peak at 4.5 to the α CH-proton and the peak at 4.0 ppm to the δ CH-protons in PTMP form II.

In both helices of PP the α -hydrogen atom is nearly in a *trans* position relative to carbonyl group and therefore the chemical shift of α CH-protons seemed to be controlled by the relative position of the preceding or the following carbonyl group. However, if the two forms of PTMP have the same conformation as PP form I and II, it is expected that the change in the chemical shift of the α CH-protons in PTMP and PP should be similar. The agreement in the downfield shifts of the forms II of PP and PTMP is, therefore, an additional evidence for their identical conformations.

Change in Reduced Viscosity During the Forward Mutarotation. Since the residue tran-

slations of PP form I (all *cis* amide bonds) and PP form II (all *trans* amide bonds) were found by X-ray diffraction to be 1.85 and 3.12 Å, respectively², transition from a *cis* to a *trans* amide structure will cause an elongation of the molecular shape and, hence, an increase in viscosity during the forward mutarotation. The viscosity change during the mutarotation of PP (I→II) in acetic acid was measured by Fasman and Blout⁸, who found that the reduced viscosity increased monotonically during the time of the forward mutarotation (0.99 to 1.44 dl/g in 4 days).

The increase in viscosity during the forward mutarotation of PTMP was also expected, if form I and II of PTMP have the same conformations as pp form I and II. The change in reduced viscosity during the forward mutarotation of PTMP (polymer No. 3 in Table 1) in TFE is shown in Fig. 7. Due to the low molecular weight of the polymer, the reduced viscosity is very low. However, the change in viscosity during the mutarotation is remarkable. Initially, the viscosity increases rapidly, and then it changes from 0.15 to 0.26 (dl/g) in five days. The rate of viscosity change is consistent with the change in uv-spectra (Fig. 3).

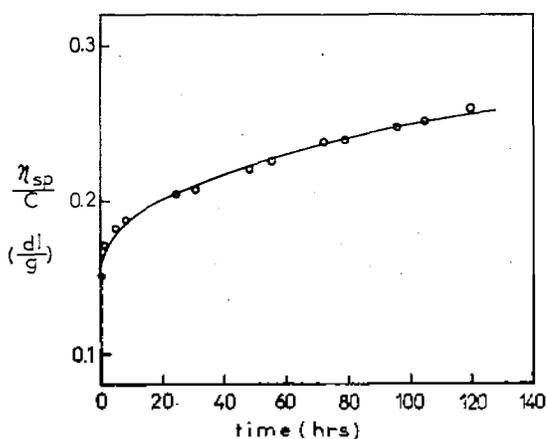


Fig. 7. Changes in reduced viscosity of PTMP during the forward mutarotation in TFE at 25°C.

This result indicates that the forward mutarotation of PTMP involves a *cis-trans* isomerization of amide bonds.

Disordered Structure in Acidic Solution.

The ordered structure of PP was destroyed by addition of a small amount of strong acid such as perchloric or hydrochloric acid¹⁰. In the CD-spectrum of PP form II in TFE-perchloric acid, the small positive band at 226 mμ disappeared, and the strong negative band at 206 mμ decreased somewhat in intensity and was red-shifted about 2 mμ. This change was accompanied by a drastic decrease in intrinsic viscosity¹⁰. Since these changes were similar to those observed for a concentrated salt solution of PP, a collapse of the PP form II helix with formation of a disordered structure was assumed.

In order to establish whether PTMP has the same solution properties in acidic solution, the CD-spectrum and intrinsic viscosity of PTMP were measured in TFE solution containing 0.05 M perchloric acid. The intrinsic viscosity value of 0.14 (dl/g) in TFE decreased to 0.08 in

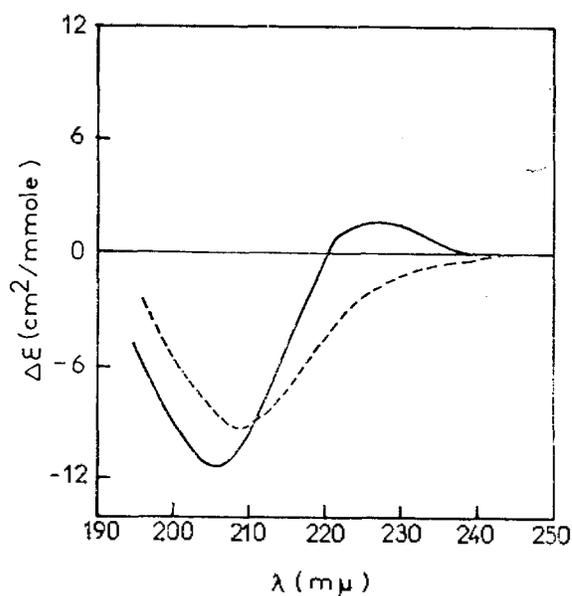


Fig. 8. CD-spectra of PTMP in TFE (solid line) and in TFE+0.055 M perchloric acid (dotted line).

TFE containing 0.05 M HClO₄. As shown in Fig. 8, the change in the CD-spectrum in acidic solution is strikingly similar to that observed for PP¹⁰. From these results it is proposed that PTMP has disordered structures in acidic solution.

Infrared Spectra of Form I and II in Solution. The two ordered structures of PP in solid state were determined by X-ray diffraction¹¹⁻¹³, whereas most physical properties and changes were studied in the solution state. It is, therefore, essential to confirm whether structures in solid state of PP are maintained in the solution state. From ORD and CD-measurements it has been concluded that these ordered structures of PP are largely maintained in solution in appropriate solvents.^{3,4,14,17} When X-ray diffraction measurements were carried out on samples prepared by precipitation of PP at the equilibrium state of form I and II, the spacing characteristic of form I and II were found.¹⁸ It was ascertained from this result that the cooperative structural transition observed by ORD and CD-spectra in solution involves a transformation between form I and II.

Ir-Spectroscopy can also be utilized for the identification of conformations in both the solid and solution states. In ir-spectra of solid PTMP the band with a maximum at 1360 cm⁻¹ is characteristic of form I, whereas the band with a maximum at 1330 cm⁻¹ is characteristic of form II¹. Both forms were dissolved in chloroform, in which PTMP does not mutarotate, and the ir-spectra were recorded (Fig. 9). Since the band positions found in this solution are exactly the same as those found for both forms in the solid state, there is little doubt that both conformations of PTMP in solution are the same as those in the solid state. Unfortunately, the solvents capable of mutarotation of PTMP (TFE and *n*-BUOH) show strong absorption

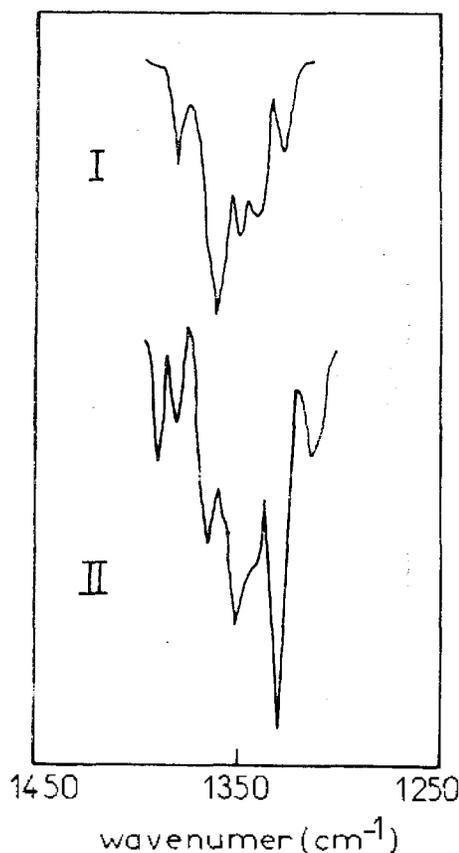


Fig. 9. ir-Spectra of PTMP form I and form II in chloroform.

bands in the 1300~1400 cm⁻¹ region. The band changes during the mutarotation could not be recorded because of disturbance of those solvent bands.

CONCNCCLUSION

The forward mutarotation of PTMP occurred in strong organic acids and TFE, while the reverse mutarotation resulted upon dilution of the TFE solution with an excess of aliphatic alcohols. The spectra of form I were converted to the spectra of form II by the forward mutarotation, in which the following changes in CD, ORD and uv-spectra were accompanied: a decrease in intensity and red-shift of the positive

band, an increase in intensity and red-shift of the negative band at $202\text{ m}\mu$, and disappearance of the negative band at $234\text{ m}\mu$ in the CD-spectra; a decrease and red-shift of trough in the ORD-spectra; a decrease in extinction and a blue-shift of the absorption band in the uv-spectra. All changes in CD, ORD and uv-spectra for the reverse mutarotation are found to be opposite to the changes during the forward mutarotation.

These trends in CD, ORD-spectra for the forward mutarotation seem to be characteristic for the transition of form I to form II of polyproline derivatives, since they are parallel to the changes in CD and ORD-spectra during the forward mutarotation of PP.

The forward mutarotation of PTMP also accompanied by changes in nmr-spectra and viscosity. The αCH peak of form II was shifted downfield about 0.3 ppm in comparison with the chemical shift of form I. An increase in viscosity, which is typical for *cis-trans* transition of amide bonds in peptide, was found during the forward mutarotation of PTMP.

The forward mutarotation of PTMP in TFE completed in 5 days, whereas the forward mutarotation of PP in the same solvent completed in 19 hours⁴. The decrease in rate of mutarotation of PTMP is due to the steric hinderance between methyl and carbonyl groups during the mutarotation of PTMP.

All changes of solution properties observed in this investigation are similar to those found for PP. These results support the proposed conformations of PTMP as the same conformations

of PP form I and form II, *i.e.*, a right-handed helix with all *cis* amide bonds and a left-handed helix with all *trans* amide bonds.

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