Studies on Cyclic Peptides. III. The Specific Interaction of Cyclic Peptides With Benzene and an Application of the Solvent-Induced Nmr Shift to the Conformational Analysis of Cyclo-(Pro-Sar-Gly)₂

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Synopsis

The interaction between cyclic peptides [cyclo-(Sar₄), cyclo-(Pro-Sar-Gly)₂, cyclo-(Sar-Sar), and cyclo-(Sar-Gly)] with benzene has been investigated by nmr spectroscopy. The experiment with cyclo-(Sar₄) showed that benzene interacted preferentially with the *trans* peptide bond in a similar manner to the dimethylformamidebenzene interaction. The solvent-induced nmr shift was then applied to the conformational analysis of cyclo-(Pro-Sar-Gly)₂ with the aid of the molecular model. The major conformation was proved to possess the C₂ symmetry with internally hydrogenbonded glycine residues, in which all peptide bonds were *trans*. The interaction of cyclo-(Sar-Sar) and cyclo-(Sar-Gly) with benzene was also studied. The association constant was 0.115-kg solution per mole of cyclo-(Sar-Sar) and 0.089-kg solution per mole of cyclo-(Sar-Gly) in chloroform.

INTRODUCTION

Some papers have been published on the intermolecular interaction between aromatic compound and peptide. They include a stereospecific collision complex of cyclo-(L-Pro₃) with benzene, which was formed in CD_2Cl_2 by the attractive interactions between the electron-rich aromatic ring and the electron-deficient H_{α} and nitrogen atoms of the peptide backbone assuming all *cis* conformation.¹ However, cyclic peptides consisting of *trans* peptide bonds showed a marked difference; with cycloheptapeptide evolidine² and cyclo-(Gly-Glu(OBzl)-Gly)₂³ all of the signals in nmr spectra shifted slightly to the lower magnetic field upon the addition of benzene $d_6(C_6D_6)$, suggesting no complex formation or the formation of a molecular complex in which benzene does not face the peptide.

Since in the above instances the experiments were performed in a dimethylsulfoxide- $d_6(C_6D_6)$ mixture, the weak solute-solvent interaction might have been destroyed by the polar solvent. In this respect, it is very interesting to investigate the interaction with benzene of cyclic peptides containing *trans* peptide bonds as well as *cis* peptide bonds in an inert sol-

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vent. Cyclic peptides consisting of sarcosine are more soluble in nonpolar solvent and suitable for the present purpose. In the present study, interactions of cyclo-(Sar₄) were investigated.

The nmr shift induced by the benzene dilution has been used to distinguish a *cis* peptide bond from a *trans* peptide bond in linear molecules.⁴⁻⁷ If the principle of the aromatic solvent-induced shift is applicable to cyclic peptides, the conformational analysis of cyclic peptides will be possible. In the present investigation, the conformational analysis of cyclo-(Pro-Sar-Gly)₂ on the basis of the solvent-induced shift and a molecular model construction was performed. The interactions of cyclo-(Sar-Sar) and cyclo-(Sar-Gly), consisting of only a *cis* peptide bond, with benzene were also investigated. The experimental findings are described in this paper.

EXPERIMENTAL

Materials

The syntheses of cyclo-(Pro-Sar-Gly)₂ and cyclo-(Sar₄) have been described in the previous paper.⁸ Cyclo-(Sar-Gly) was synthesized from sarcosylglycine benzyl ester hydrochloride⁸ in ammonia-methanol. Recrystallization from ethyl acetate gave white needles; yield 82%; mp 147°-148°C (Ref. 9, 139°-140°C). Cyclo-(Sar-Sar) was synthesized as described previously.¹⁰ Deuterated compounds used were of reagent grade. Benzene and chloroform were purified in the usual manner¹¹ and distilled before use.

Procedures

Nmr spectra were recorded on a Varian HA-100 and HR-220 spectrometer. A double-resonance experiment was accomplished using a Hewlett Packard 4204A digital oscillator. Tetramethylsilane(TMS) was used as a reference and an internal rock at 100 MHz. Temperature-variable experiments were carried out using ethylene glycol for the calibration on the Varian charts. Circular dichroism(CD) was measured on a Jasco J-20 spectrometer.

Analytical Method

The association constant K for the following equilibrium can be estimated from the nmr study, where A and D represent an acceptor molecule (cyclic

$$A + D \xrightarrow{K} AD$$

peptide) and a donor molecule (benzene), respectively.

If the "1:1" complex is formed, and the exchange of complexed molecule with uncomplexed molecule is rapid on the nmr time scale, the following relationship is obtained under the condition $[D] \gg [A]$ according to Foster,¹²

$$\Delta/[D] = \Delta_0 \cdot K - \Delta \cdot K. \tag{1}$$

where Δ is the difference of the chemical shift of a given proton of A in the presence and in the absence of D, and Δ_0 is the difference of the same proton between the "1:1" complex and the uncomplexed A. A plot of $\Delta/[D]$ against Δ may give the association constant K and Δ_0 .

RESULTS AND DISCUSSION

Interaction of Cyclo-(Sar₄) With Benzene

Cyclo- (Sar_4) has been reported to be centrosymmetric with the *cis-trans*cis-trans sequence of the peptide bond.^{13,14} The effect of benzene- d_6 on the nmr spectra of cyclo- (Sar_4) is shown in Figure 1. Figure 2 shows the shift of the nmr signals as a function of the mole fraction of benzene- d_6 . All of the signals shifted to the upper magnetic field on increasing the mole fraction of C_6D_6 . The cyclic peptide is so rigid that the conformational change is not induced by the solvent and cannot explain the shift. The shift was thus caused by the formation of an intermolecular complex. The extent of the up-field shift decreased in the order $CH_2(d)(e) > trans N$ - $CH_3(a) > cis N-CH_3(b) > CH_2(c)(f)$. It should be noted that benzene diamagnetically shielded the sarcosine N-methyl group (a), and even more strongly affected the sarcosine α -proton (d)(e) resonances, indicating that it interacts preferentially with the *trans* peptide bond of $cyclo-(Sar_4)$. From these experimental facts the average geometry of the complex is supposed to be such that benzene is situated obliquely over the peptide backbone, facing its plane to the trans N-CH₃(a) and the CH₂(d)(e) protons as shown in Figure 3. The electron-rich benzene plane is attracted by the partial positive charge on the nitrogen atom, but repelled by the partial negative charge on the carbonyl oxygen atom of the trans peptide group. On the other hand, the N-methyl group of the cis peptide bond does not interact with benzene as strongly as that of the *trans* peptide bond, because an approach of benzene to the methyl group causes a repulsion by the negatively charged carbonyl oxygen of the *cis* peptide bond. This is quite similar to the model proposed for the DMF-benzene complex,⁵ but different from that proposed for the diketopiperazine-benzene complex, which will appear subsequently.

These results suggest that the solvent-induced nmr shift is applicable to distinguish the *cis-trans* isomerism of the peptide bond of cyclic peptides (except for diketopiperazine) as well as simple amides and linear oligopeptides. It should be emphasized here, however, that the steric factors of cyclic peptides should be considered when one analyzes the conformation of cyclic peptides according to the above method.

Conformational Analysis of Cyclo-(Pro-Sar-Gly)₂

220-MHz nmr spectra of cyclo-(Pro-Sar-Gly)₂ are shown in Figure 4. The nmr spectrum in CDCl₃ was analyzed as follows. Protons of glycine residue were assigned by the spin decoupling method and analyzed as ABX spin systems. α -, β -, γ -, and δ -protons of proline residue were assigned in a



Fig. 1. 100-MHz nmr spectra of cyclo-(Sar₄). (A)—In CDCl_3 , (B)— $\text{CDCl}_3-\text{C}_6\text{D}_6$ (3:1); (C)— $\text{CDCl}_3-\text{C}_6\text{D}_6(1:1)$. Concentration: 1.5-2 mg/ml, signals (a)-(f) correspond to the protons of Fig. 3.

similar way using the double-resonance method. A large splitting of the AB-type spectrum was observed for α -methylene protons of the sarcosine residue, suggesting the difference of the proton environment. These assignments were confirmed by comparison with the spectra of the intermediate products during synthesis, and other cyclic peptides containing sarcosine or proline. Except for two minor resonances at δ 2.93 and 3.24



Fig. 2. Chemical shift of the protons of cyclo-(Sar₄) as a function of the mole fraction of benzene- d_6 . Concentration: 0.7-2 mg/ml; for (a)-(g), see Figs. 1 and 3.



Fig. 3. Average geometry of cyclo-(Sar₄)-benzene complex. Protons (a)-(f) correspond to the signals in Figs. 1 and 2. A benzene molecule approaching to the second *trans* N-CH₃ group is not shown because of the centrosymmetry of the complex.

ppm of N-CH₃ protons of sarcosine residue, only one set of signals was observed for the individual amino acid residue in CDCl₃. This implies that cyclo-(Pro-Sar-Gly)₂ possesses C₂ symmetry in CDCl₃ on the nmr time scale.

It may be expected that $cyclo-(Pro-Sar-Gly)_2$ assumes two or more conformations because the *cis* peptide bond of the sarcosine residue is stable as well as proline.^{15,16} It is acceptable that the cyclohexapeptide assumes two different conformations in CDCl₃, and this is evidently the case in methanol and DMSO-d₆ as shown in Figure 5. On going from CDCl₃ to methanol and finally to DMSO-d₆, the two minor resonances (h) of N-CH₃ protons of the sarcosine residue increased the peak area, while the major resonance (H) of N-CH₃ protons decreased. Moreover, three resonance signals were observed with the Gly-NH proton in DMSO-d₆. The two minor resonances (h₁, h₂) having an equal intensity appeared in accompani-



Fig. 4. 220-MHz nmr spectra of cyclo-(Pro-Sar-Gly)₂. (A)—In CDCl_3 ; (B)--CDCl₃-C₆D₆(3:1); (C)--CDCl₃-C₆D₆(1:1). Concentration: 20-25 mg/ml.

ment with the increase of the peak area of the minor resonance signals (h) for Sar-N-CH₃ protons. These resonance signals are supposed to reflect a minor conformation of the cyclohexapeptide. They are not due to impurities, because the nmr spectrum of the cyclohexapeptide did not change even after repeated recrystallizations and the three NH resonance signals coalesced at 94°C. The two minor resonance signals for either NH or N-CH₃ protons should belong to a conformation (h), not to two or more conformations. This is supported by the fact that with either Sar-N-CH₃ or Gly-NH protons, the ratio of the two minor resonance signals were always unity in any solvent employed. From the fractional peak area h/h + H, the content of the minor conformation was determined to be 8% in CDCl₃, 12% in CD₃OD, and 24% in DMSO-d₆ at 23°C. The content was not influenced significantly by the temperature. The major conformation (H) possesses C₂ symmetry, while the minor conformation (h) is asymmetric.

The temperature dependence of the NH resonances in DMSO- d_6 and methanol is shown in Figure 6. An up-field shift of peptide proton resonance with increasing temperature is useful to distinguish an NH proton shielded from solvent.¹⁷ Since cyclo-(Pro-Sar-Gly)₂ carries no bulky sub-



Fig. 5. 100-MHz nmr spectra of cyclo-(Pro-Sar-Gly)₂ in DMSO- d_6 (upper figure) and in MeOH- d_4 (lower figure). The spectrum of the NH region of the latter was recorded in methanol. Concentration: 25 mg/ml.

stituent, solvent molecules easily approach the NH bond of the cyclic hexapeptide. Therefore, a small temperature coefficient implies an internally hydrogen-bonded NH proton. The major resonance (H) and one of the two minor resonances (h_1) were less sensitive to the temperature variation, indicating that the NH protons are involved in the internal hydrogen bonding. On the other hand, the minor resonance signal at lower magnetic



Fig. 6. The temperature dependence of the chemical shift of the NH resonances of cyclo-(Pro-Sar-Gly)₂.

field (h_2) has a temperature coefficient of 0.0021 ppm/deg in DMSO- d_6 . This peptide proton of the conformation h_2 is more exposed to the solvent than those of the conformations H and h_1 . Thus in the minor conformation (h) one of the peptide protons of glycine residue is involved in the internal hydrogen bonding, while the other is exposed to the solvent.

The nmr spectrum ascribable to the major conformation (H) in CDCl₃ is essentially the same as in methanol and DMSO- d_6 . It is therefore reasonable to assume that the C₂ conformation with internally hydrogen-bonded glycine residue (H) is retained in CDCl₃. Under all conditions employed, a triplet signal was observed for the proline α -proton resonance, which indicates the *trans* Gly-Pro peptide bond.¹⁸ If one considers this as well as the intramolecular hydrogen bonding of 1,4-type, which constructs a β turn,¹⁹ the two Sar-Gly peptide bonds should be *trans* due to the steric constraint of the pyrrolidine ring of proline residue. This is evident from the construction of the cyclohexapeptide using the space-filling (CPK) molecular model. However, the nature of Pro-Sar peptide bonds remains uncertain. The molecular model shows that either *cis* or *trans* conformation is acceptable for the Pro-Sar bond, though *cis* conformation imposes undue strains on the molecular skeleton.

To investigate this problem further, the shift of nmr signals in $CDCl_3$ nduced by the addition of benzene- d_6 was examined. In Figure 4 is shown the effect of benzene- d_6 on the nmr spectrum of cyclo-(Pro-Sar-Gly)₂. Figure 7 shows the chemical shift of various proton resonance signals as a



Fig. 7. Chemical shift of the protons of cyclo-(Pro-Sar-Gly)₂ as a function of the mole fraction of benzene- d_6 . Concentration: 8-25 mg/ml. (----O--- \rightarrow) Pro-C_{β}H₂, -) Sar-N-CH₃(h); (------) Sar-N-CH₃(H); $C_{\gamma}H_2$; (-(---) -×– Sar- $C_{\alpha}H_2$; (---0--)Pro-CH₂; (- $-\Delta$ ——)Gly-C_aH₂; (---0 -)Pro-C_{α}H; -_ -)Gly-NH.

function of the mole fraction of benzene- d_6 . It is seen that α -, β -, γ -, and δ -protons of the proline residue and the N-methyl proton (H) of the sarcosine residue shifted significantly to the upper magnetic field. The shift was moderate for α -methylene protons of the glycine residue and one of the two α -methylene protons of the sarcosine residue. The chemical shifts of the other α -methylene proton of the sarcosine residue and the peptide proton (NH) were hardly affected by the addition of benzene. Since coupling constants of NH-C_aH₂ of the glycine residue and the ratio h/h + H of N-CH₃ signals of the sarcosine residue remained unchanged, it is believed that the change of the solvent composition was not accompanied by conformational change. These experimental data, together with the discussion given below, support the suggestion that the major conformation (H) of cyclo-(Pro-Sar-Gly)₂ is C_2 -symmetric, in which all peptide bonds are trans and two glycine residues are internally hydrogen-bonded. This conformation is shown in Figure 8. Discussions on the temperature-induced shift of the minor resonance signals for $Sar-N-CH_3$ (h) will appear subsequently.



Fig. 8. Major conformation (H) of cyclo-(Pro-Sar-Gly)₂.

The above conclusion is based on the assumption that benzene interacts with the electron-deficient site of the cyclopeptide. This assumption seems to be reasonable on account of experimental results obtained with the cyclo-(Sar₄)-benzene interaction. Peptide protons (NH) of fully extended glycine residues cannot interact with benzene since they are buried in the peptide backbone due to the intramolecular hydrogen bonding. One of the α -methylene protons of the sarcosine residue projects perpendicularly out of the plane of the peptide backbone and is located near to the partially negative carbonyl oxygen of the trans Pro-Sar peptide bond. This side of the cyclic peptide, the lower side of Figure 8, is electronegative, so benzene would not be accessible to the protons on this side. The other methylene proton, N-methyl protons of the sarcosine residue, and the α -proton of the proline residue are situated at the opposite side of the carbonyl oxygen of the trans Pro-Sar peptide bond. This side of the cyclic peptide, the upper side of Figure 8, is electropositive. Thus benzene should easily approach the protons involved in the upper side of the cyclic peptide, inducing the up-field shift of the resonance signals for these protons.

If the conformation of the Pro-Sar peptide bond were *cis*, the α -proton of the proline residue and *N*-methyl protons of the sarcosine residue would be brought into the proximity of the carbonyl oxygen of the *cis* Pro-Sar peptide bond. Under this condition, the approach of benzene to these protons is prohibited and little nmr shift would have been observed with these protons on the addition of C₆D₆. This consideration gives support to the idea that in the major conformation of cyclo-(Pro-Sar-Gly)₂ the Pro-Sar peptide bond assumes a *trans* conformation.

The complete analysis of the minor conformation (h) of the cyclic peptide was not attained. However, it is safe to say that this conformation is asymmetric, having one of the two NH protons exposed to the solvent, and the other internally hydrogen-bonded. Since the resonance signal for the sarcosine N-CH₃ group in the minor conformation appears as a doublet, the cis-trans isomerization around the Pro-Sar peptide bond seems to be probable. One of the two Pro-Sar peptide bonds could be cis and the other trans, because the extent of the shift of the minor N-methyl resonance signals on the addition of C_6D_6 was different; the up-field shift of the resonance signal at higher magnetic field was greater than that at lower magnetic field.

The circular dichroism (CD) spectrum of cyclo-(Pro-Sar-Gly)₂ was measured in methanol. It showed a positive Cotton effect at 210 nm ($[\theta] =$ +189000) and a negative Cotton effect at 238 nm ($[\theta] = -54000$). The related compound, cyclo-(Pro-Gly-Gly)₂ has been reported²⁰ to show a positive Cotton effect at 201 nm ($[\theta] = +11000$) and a negative Cotton effect at 225 nm ($[\theta] = -2500$). Apart from the shift of the absorption maxima and the magnitude of the circular dichroism, the overall pattern of the CD spectra was similar, indicating the similar conformation for the two cyclohexapeptides. It has been reported too that cyclo-(Pro-Gly-Gly)₂ assumes the same conformation^{21,22} as that depicted for cyclo-(Pro-Sar-Gly)₂ in Figure 8. The CD spectra were, however, quite different from those of cyclo-(Pro-Pro) and cyclo-(Pro₃).²⁰

Interaction of Diketopiperazine With Benzene

The nmr spectra of cyclo-(Sar-Sar) and cyclo-(Sar-Gly) are shown in Figure 9. The peptide backbone of cyclo-(Sar-Sar) has been proposed to assume a planer or a rapidly converting conformation.²³

In the spectrum of cyclo-(Sar-Gly) two kinds of α -methylene signals are observed, one of which is a broad singlet and the other is an AB quartet. The assignment was made as shown in the insert of Figure 9. The singlet at the lower magnetic field was broadened on the addition of di-*tert*-butyl nitroxide.²⁴ This indicates the occurrence of a hydrogen bonding between the radical and a peptide proton (NH).²⁵ Therefore the peak at the lower magnetic field was assigned to the C_{α}H₂ protons of glycine residue and the higher magnetic field peak to those of the sarcosine residue. From the small AB quartet of C_{α}H₂ of the sarcosine residue in Figure 9, it was considered that the diketopiperazine ring of cyclo-(Sar-Gly) deviates slightly from the planarity.

It is seen in Figure 9 that all of the nmr signals shifted to up-field on increasing the mole fraction of benzene in the mixed solvent, suggesting the formation of a molecular complex.

As shown in Figure 10, the plot of $\Delta/[D]$ against Δ gave a straight line indicating the formation of a "1:1" complex. The association constant Kwas determined from the slope according to Eq. (1) and is given in Table I. The K values are comparable to that of DMF²⁶ in cyclohexane (0.13 kgsolution/mol at 37.5°C) and to that of cyclo-(Gly-Gly)²⁷ in water [1.0 (mole fraction)⁻¹ at 0°Cl.

From the Δ_0 values, the average geometry of the weak molecular complex can be determined according to the Johnson-Bovey's ring-current contribution.²⁸ Usually five parameters are necessary to specify the geom-



Fig. 9. The effect of benzene on the nmr spectra of cyclo-(Sar-Sar) and cyclo-(Sar-Gly). (A)—Cyclo-(Sar-Sar) in CHCl₃-C₆H₆; (B)—cyclo-(Sar-Gly) in CDCl₃-C₆D₆.

TABLE	Ι
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Association Constant and Δ_0 for Diketopiperazine–Benzene Complexes ^a							
	Benzene						
	[D]	[D] KAT	_у ь				
	(mol/kg~	(kg-solution/	[(mol	$\Delta_0^{CH_3}$	$\Delta_0^{\mathbf{CH}_2}$		
	solution)	solution) mol) $frac.)^{-1}$	frac.) ⁻¹]	(Hz)	(Hz)		
Cyclo-(Sar-Sar)	2.366-6.585	0.115 ± 0.009	0.97 ± 0.04	127 ± 10	160 ± 18		
Cyclo-(Sar-Gly)	0.937 - 6.223	0.089 ± 0.006	0.69 ± 0.03	166 ± 20	215 ± 26		

* 100 MHz, $[A] = 0.022 - 0.031 \text{ mol/kg-solution}, 31.5^{\circ}\text{C}.$

^b Average value for N-methyl and α -methylene protons.



Fig. 10. Plots of $\Delta/[D]$ against Δ for cyclo-(Sar-Sar)-benzene complex (A) and cyclo-(Sar-Gly)-benzene complex (B).

etry of a "1:1" complex.²⁹ However, the geometry of the cyclo-(Sar-Sar)-benzene complex was determined reasonably from $\Delta_0^{\text{CH}_3}$ and $\Delta_0^{\text{CH}_2}$. The benzene ring lies over the diketopiperazine ring as a result of dipoleinduced dipole interactions. This interaction occurs between two amide dipoles and the π -electron cloud of benzene. The two ring planes are nearly parallel with a distance of about 2.2–2.6 Å. The distance is somewhat smaller than the van der Waals contact of the complex (2.80–2.85 Å). The disagreement was caused by the neglect of the effect of chloroform on the interaction. If the contribution of the solvent shell³⁰ had been taken into account, K values would have been greater and Δ_0 smaller.

The intermolecular interaction of benzene with cyclic peptides was found to be weak. However, in some cyclic peptides containing aromatic amino acid residues, the intramolecular interactions of the aromatic substituent with the peptide bond should be strong enough to have a significant effect on the conformational stability.^{31,32} This could be achieved from a lesser entropy loss in the interactions when the interacting groups are placed favorably for the intramolecular interactions.

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References

- 1. Torchia, D. A. & Deber, C. M. (1972) Biopolymers 11, 653-659.
- 2. Kopple, K. D. (1971) Biopolymers 10, 1139-1152.
- 3. Sugihara, T. (unpublished result).
- 4. Hatton, J. V. & Richards, R. E. (1960) Mol. Phys. 3, 253-263.
- 5. Hatton, J. V. & Richards, R. E. (1962) Mol. Phys. 5, 139-152.
- 6. La Planche, L. A. & Rogers, M. T. (1964) J. Amer. Chem. Soc. 86, 337-341.

7. Madison, V. & Schellman, J. (1970) Biopolymers 9, 511-567.

8. Sugihara, T., Imanishi, Y. & Higashimura, T. (1975) Biopolymers 14, 723-731.

9. Poddubnaya, V. A. & Lavrenova, G. I. (1958) Vitnik Moskov Univ., Ser. Mat., Mekh. Astron., Fiz., Khim. 13, 165-175.

10. Sugihara, T., Imanishi, Y. & Higashimura, T. (1973) Biopolymers 12, 2823-2831.

11. Riddick, J. A. & Bunger, W. B. (1970) Organic Solvents, Wiley-Interscience, New York, Ch. V.

12. Foster, R. (1960) Organic Charge-Transfer Complexes, Academic, New York, pp. 140-147.

13. Dale, J. & Titlestad, K. (1970) Chem. Commun. 1403-1404.

14. Titlestad, K., Groth, P. & Dale, J. (1973) Chem. Commun., 646-647.

15. Bovey, F. A., Ryan, J. J. & Hood, F. P. (1968) Macromolecules 1, 305-307.

16. Howard, J. C., Momany, F. A., Andreatta, R. H. & Scheraga, H. A. (1973) Macromolecules 6, 535-541.

17. Urry, D. W. & Ohnishi, M. (1970) Spectroscopic Approaches to Biomolecular Conformation, Amer. Med. Ass., Chicago, Ill., Ch. VII, pp. 263–303.

18. Patel, D. J. (1973) Biochemistry 12, 667-676.

19. Kopple, K. D., Go, A., Logan, Jr., R. H. & Savrda, J. (1972) J. Amer. Chem. Soc. 94, 973-981.

20. Deber, C. M., Scatturin, A., Vaidya, V. M. & Blout, E. R. (1970) Peptides; Chem. Biochem. Proc. Amer. Peptide Symp., 1968, pp. 163-173.

21. Schwyzer, R. & Ludescher, U. (1969) Helv. Chim. Acta 52, 2033-2040.

22. Pease, L. G., Deber, C. M. & Blout, E. R. (1973) J. Amer. Chem. Soc. 95, 258-260.

23. Dale, J. & Titlestad, K. (1969) Chem. Commun. 656-659.

24. Morishima, I., Endo, K. & Yonezawa, T. (1971) J. Amer. Chem. Soc. 93, 2048-2050.

- 25. Morishima, I., Ishihara, K., Inubushi, T. & Yonezawa, T. (in preparation).
- 26. Sandval, A. A. & Hanna, M. W. (1966) J. Phys. Chem. 70, 1203-1206.
- 27. Kopple, K. D. & Marr, D. H. (1967) J. Amer. Chem. Soc. 89, 6193-6200.
- 28. Johnson, Jr., C. E. & Bovey, F. A. (1958) J. Chem. Phys. 29, 1012-1014.

29. Baldwin, J. E. (1965) J. Org. Chem. 30, 2423-2425.

30. Foster, R. & Fyfe, C. A. (1966) Trans. Faraday Soc. 62, 1400-1405.

31. Chandrasekaran, R., Lakshminarayanan, A. V., Mohanakrishnan, P. & Ramachandran, G. N. (1973) *Biopolymers* 12, 1421-1425.

32. Vicăr, J., Buděšínský, M. & Bláha, K. (1973) Collection Czech. Chem. Commun. 38, 1940–1956.

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