Chemical Studies on Tuberactinomycin. IX.[1] Nuclear Magnetic Resonance Studies on Tuberactinomycins and Tuberactinamine N[2]

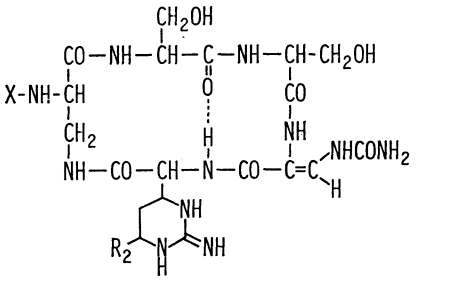
Tateaki WAKAMIYA and Tetsuo SHIBA

Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560

(Received May 12, 1975)

Conformational analyses of peptide antibiotics tuberactinomycins were performed by NMR spectroscopy of 100 MHz. Studies on temperature dependence and deuterium exchange in amide proton resonances revealed the presence of an intramolecular hydrogen bond participated with α-amide proton of the guanidino amino acid residue in the cyclic part of each molecule. The same result was obtained in tuberactinamine N, which is the cyclopeptide moiety without amino acid side chain in tuberactinomycins N and O. The common rigid structure fixed by hydrogen bonding in the cyclic part seems to be a requisite for the exhibition of microbial activity in tuberactinomycin family.

The total chemical structures of antituberculous peptides tuberactinomycins A, B, N, and O, which were isolated from *Streptomyces griseoverticillatus* var. *tuberacticus*, were determined in our previous investigations as shown in Fig. 1.[3-8] The steric structure of tuberactinomycin O, one of the four congeners, was established by means of X-ray analysis.[4] Subsequently, Bycroft *et al.* studied on X-ray analysis of viomycin (tuberactinomycin B), applying our informations on tuberactinomycin O that the monohydrochloride-dihydrobromide of the antibiotic is adequate to crystallographic investigation.[9] From these studies, the presence of an intramolecular hydrogen bond between the α-amide proton of the guanidino amino acid residue, *i.e.*, capreomycidine (Cpd) or tuberactidine (Tbd), and the carbonyl oxygen of the serine residue (Ser(1)) neighboring the diaminopropionic acid (Dpr) residue was found in tuberactinomycin O and viomycin. This intramolecular hydrogen bond seems to be very important from the viewpoint of a relationship between the conformations of tuberactinomycins and their antimicrobial activities.



X=$CH_2CH_2CH(NH_2)$–$CH(R_1)$–CH_2CO–

Tuberactinomycins		
A	R_1=OH,	R_2=OH
B	R_1=H,	R_2=OH
N	R_1=OH,	R_2=H
O	R_1=H,	R_2=H
Tuberactinamine N	X=H,	R_2=H

Fig. 1. Chemical structures of tuberactinomycins and tuberactinamine N.

For studies on the structure of cyclic peptide, particularly related to the intramolecular hydrogen bond, NMR is known to be highly informational. In fact, many NMR studies on biologically active cyclopeptides, *e.g.*, gramicidin S,[10] oxytocin,[11] lysine vasopressin,[11d-e,12] and stendomycin,[13] have been reported. In the present investigation, the steric conformations of four tuberactinomycins in solutions were analyzed by their NMR spectra of 100 MHz. Furthermore, we recently succeeded in isolating a cyclic peptide moiety called tuberactinamine N (Fig. 1) common to molecules of tuberactinomycins N and O by selective liberation of γ-hydroxy-β-lysine residue from tuberactinomycin N with acid treatment.[1] Tuberactinamine N was found to possess comparable antibacterial activities to those of the mother molecule against several strains of tubercule bacilli.[1] NMR study on four tuberactinomycins and one tuberactinamine indicated clearly the presence of the same intramolecular hydrogen bond in all compounds.

From the results these conformational analyses in solutions referring to the crystal structures of tuberactinomycins O and B, we assumed that the conformation of the cyclic peptide, particularly the presence of the intramolecular hydrogen bond in the cyclic part, may reflect to the biological activities of those antibiotics as a requisite.

Experimental

Spectra were recorded on a Varian Associates XL-100-15 spectrometer equipped with a Varian VFT-100 (620/L) computer. Sample solutions were measured in tubes of 5 mm o.d. and the temperature was normally kept at 37 °C. The spectra were taken with internal H^2 lock method, thus in the case of H_2O solution 20% of DMSO-d_6 being added. Chemical shifts were obtained by δ value (ppm) from DSS in aqueous media and from TMS in DMSO-d_6 solution. Proton double resonances were carried out with frequency-sweep decouplings.

Materials. The samples of tuberactinomycins A, N, and O were prepared as trihydrochloride, whereas tuberactinomycin B (viomycin) was crystallized as monohydrochloride–dihydrobromide. Tuberactinamine N dihydrochloride was obtained by the selective cleavage of γ-hydroxy-β-lysine residue from tuberactinomycin N with concentrated hydrochloric acid according to the mechanism of *N,O*-acyl migration reaction.[1] Sample concentrations in NMR measurements were 15% in D_2O (also in the case of D_2O+TFA), 10% in H_2O (pH 2.5), and 4% in DMSO-d_6.

Results and Discussion

Spectral Assignments. In the PMR spectra of tuberactinomycins in D_2O solution, some α-methine protons were concealed by HOD signal. However, on addition with trifluoroacetic acid to the solution, those protons manifested themselves entirely with the shift of HOD signal to a lower field by about 0.7 ppm. This effect enabled decoupling experiments, and thereby spectral assignments covering all protons became feasible. Since the differences in the chemical structures of tuberactinomycin family are only limited to the degree of hydroxylation at R_1 and R_2 as shown in Fig. 1, the patterns of the corresponding signals in their spectra show close resemblances to each other. Thorough comparisons in spectra of four tuberactinomycins and one tuberactinamine were very profitable for the complete assignments of all signals. Especially a relatively simple spectrum of tuberactinamine N (Fig. 2) without the branched amino acid residue gave many key informations for assignments in tuberactinomycins (Fig. 3).

In the molecule of tuberactinamine N, there are five methylene groups of three types, *i.e.*, –C–CH_2–C–, –NH–CH_2–C–, and –C–CH_2–OH. Of these groups, γ-methylene protons in six-membered pyrimidine ring of Cpd appeared at around δ 1.8 and 2.1 as non-equivalent multiplets. A multiplet of δ-methylene protons of Cpd were resonated at δ 3.32 that corresponds to normal position for –NH–CH_2–C– type methylene. On the contrary, one of two β-methylene protons of Dpr showed normal chemical shift at δ 3.30, while the another appeared at an anomalous downfield of δ 4.12 as a quartet. This assignment was confirmed by the decoupling experiment in which an irradiation at δ 3.30 caused the quartet at δ 4.12 to collapse to a doublet and *vice versa*, accompanying concurrent decoupling with α-methine proton of Dpr at δ 4.40. Such large difference between two chemical shifts of non-equivalent β-methylene protons of Dpr seems to be ascribed to an environmental difference which comes from the situation that the peptide backbone in the cyclic moiety may be fixed in a definite conformation and prohibited from free rotation.

β-Methylene protons of two serine residues showed also significant differences in their chemical shifts. One residue Ser(1) connected with Dpr appeared at δ 3.95 as a doublet coupled with a triplet at δ 4.84, while the other Ser(2) adjacent to 3-ureidodehydroalanine (Uda) residue showed double-doublets at δ 3.90 and 4.20, respectively. Thess different situations indicate that some neighboring effect may act on the latter group causing a magnetic non-equivalency of two protons likely as in the case of β-methylene of Dpr.

Although the region from δ 4.0 to 4.5 was somewhat complicated by overlapping of five protons, the decoupling study and the comparison of coupling constants enabled the complete assignment. Two quartets at δ 4.12 and 4.20 were already assigned to one of β-methylene protons of Dpr and Ser(2), respectively. A quartet centered at δ 4.32 can be assigned to α-methine proton of Ser(2) as this has the same coupling constant (J=5 Hz) with a quartet at δ 4.20. An irradiation of a quartet centered at δ 4.40 caused a multiplet at δ 3.30 to collapse to a single peak, thereby this quartet was assigned to α-methine proton of Dpr. A multiplet at

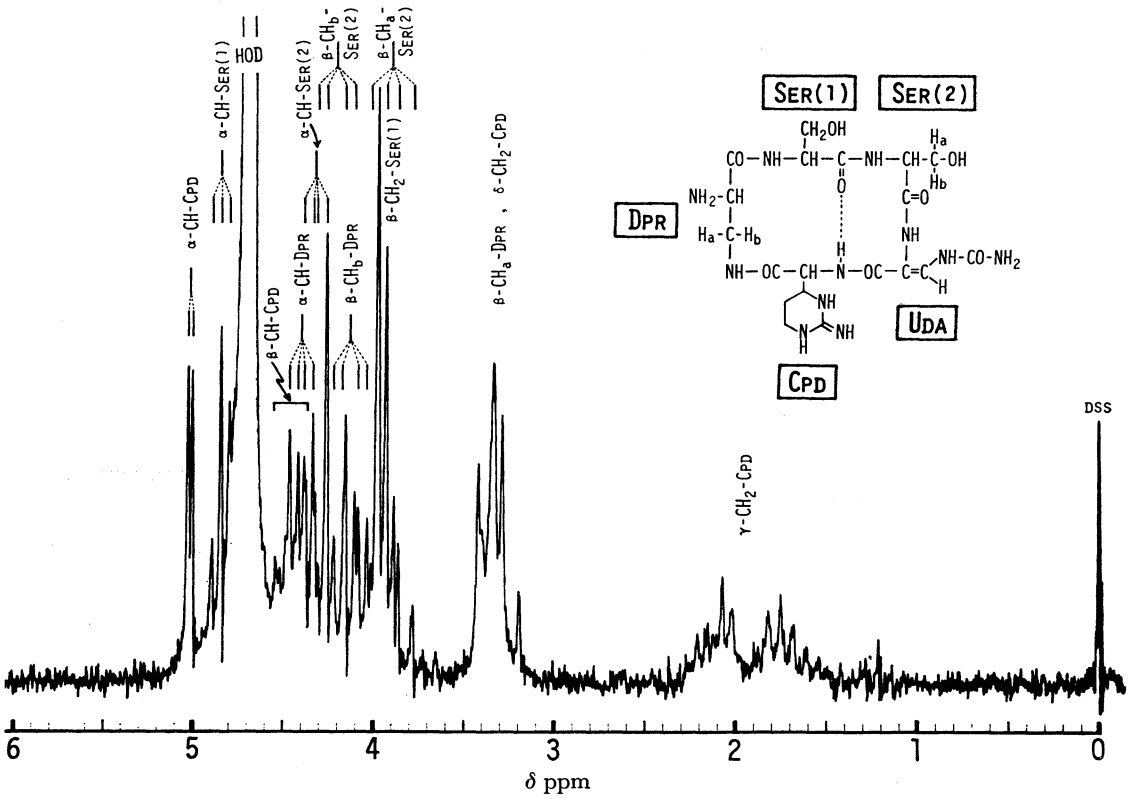


Fig. 2. NMR spectrum of tuberactinamine N in high-field region in D_2O.

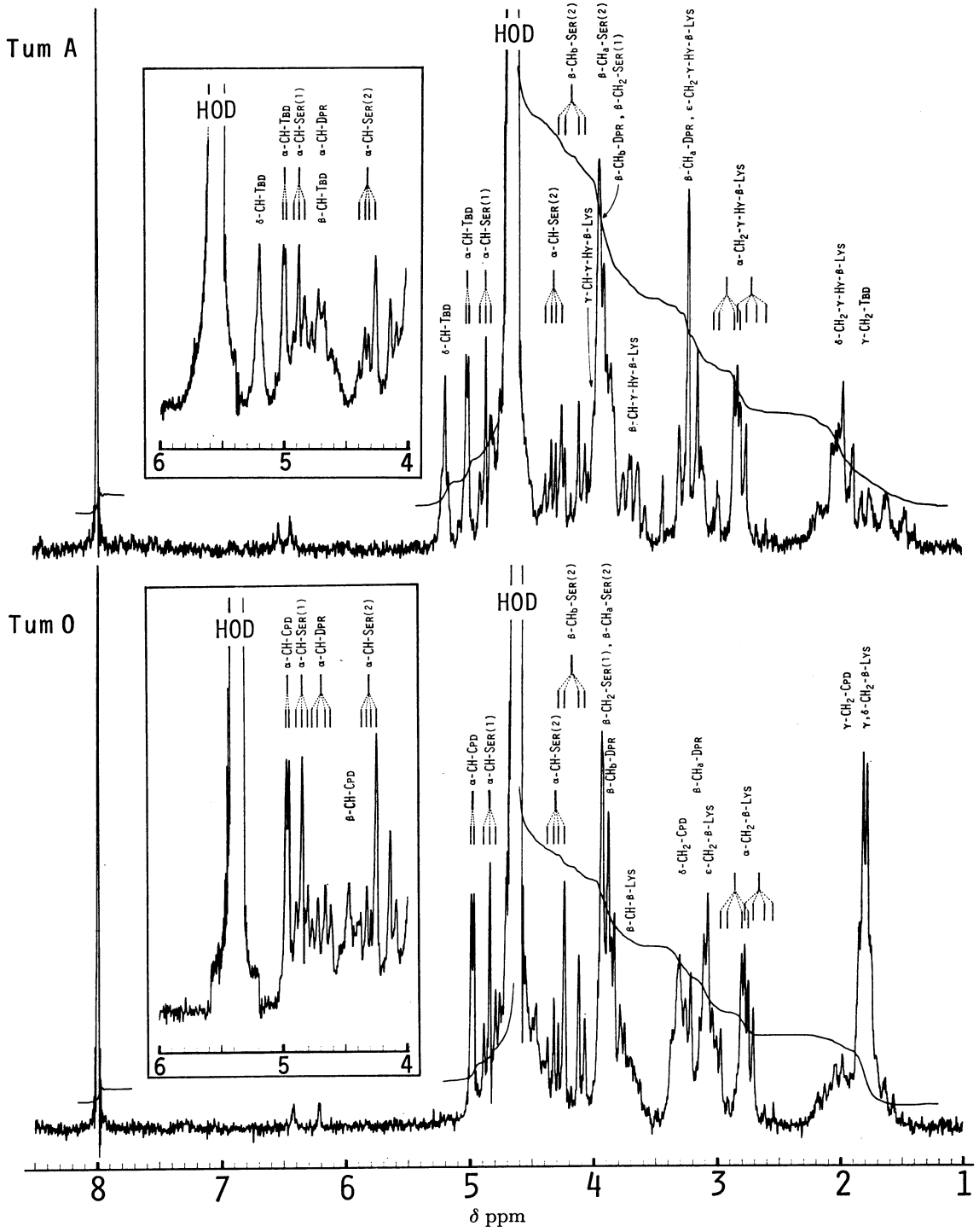


Fig. 3. NMR spectra of tuberactinomycins (Tum) A and O in D_2O. Figures in squares indicate the parts of spectra in D_2O+TFA.

δ 4.35 to 4.55 was collapsed by an irradiation of methylene protons at Cpd at δ 1.80 and 2.10. Reversely, an irradiation at δ 4.45 caused not only a change of Cpd γ-methylene as mentioned above but also of doublet at δ 5.01 of Cpd α-proton both to singlets. Thus the multiplet centered at δ 4.45 was determined to be due to β-methine proton of Cpd. A triplet at δ 4.84 and a doublet at 5.01 were necessarily assigned to α-methine protons of Ser(1) and Cpd, respectively as mentioned above already. Finally, a sharp peak at δ 8.04 was due to an olefinic proton of Uda, –C=CH–NHCONH$_2$.

Since the molecules of tuberactinomycins N and O are built up by joinings of γ-hydroxy-β-lysine and β-lysine respectively into the free α-amino group of Dpr in tuberactinamine N, simple comparisons of their spectra with that of tuberactinamine N are good enough for their assignments. However, by the effect of joining of β-amino acid with α-amino group of Dpr in tuberactinomycin N or O, the signal of the α-methine of the

TABLE 1. CHEMICAL SHIFTS OF TUBERACTINOMYCINS AND TUBERACTINAMINE N IN D_2O

		Tuberactinomycins				Tuberactinamine N
		A	B	N	O	
β-Lys	α-CH_2		2.66(1H, dd)		2.65(1H, dd)	
			2.86(1H, dd)		2.85(1H, dd)	
	β-CH		3.7 (1H, m)		3.7 (1H, m)	
	γ-CH_2		1.8 (2H, m)		1.8 (2H, m)	
	δ-CH_2		1.8 (2H, m)		1.8 (2H, m)	
	ε-CH_2		3.08(2H, m)		3.07(2H, m)	
γ-Hy-β-Lys	α-CH_2	2.72(1H, dd)		2.68(1H, dd)		
		2.92(1H, dd)		2.89(1H, dd)		
	β-CH	3.7 (1H, m)		3.7 (1H, m)		
	γ-CH	3.95(1H)		3.9 (1H)		
	δ-CH_2	1.95(2H, m)		1.95(2H, m)		
	ε-CH_2	3.22(2H, t)		3.20(2H, t)		
Dpr	α-CH	4.7 (1H)[a]	4.7 (1H)[a]	4.73(1H, q)[a]	4.70(1H, q)[a]	4.40(1H, q)
	β-CH_2	3.2 (1H)	3.1 (1H)	3.2 (1H)	3.1—3.3(1H)	3.30(1H)
		3.95(1H)	3.85(1H)	3.9 (1H)	3.9 (1H)	4.12(1H, q)
Ser(1)	α-CH	4.86(2H, t)	4.85(1H, t)	4.84(1H, t)	4.85(1H, t)	4.84(1H, t)
	β-CH_2	3.95(2H)	3.9 (2H)	3.9 (2H)	3.9 (2H)	3.95(2H, d)
Ser(2)	α-CH	4.32(1H, q)	4.31(1H, q)	4.31(1H, q)	4.30(1H, q)	4.32(1H, q)
	β-CH_2	3.95(1H)	3.9 (1H)	3.9 (1H)	3.9 (1H)	3.90(1H, dd)
		4.17(1H, dd)	4.15(1H, dd)	4.16(1H, dd)	4.18(1H, dd)	4.20(1H, dd)
Uda	β-CH	8.00(1H, s)	8.00(1H, s)	7.99(1H, s)	8.00(1H, s)	8.04(1H, s)
Cpd	α-CH			4.97(1H, d)	4.98(1H, d)	5.01(1H, d)
	β-CH			4.5 (1H, m)[a]	4.5 (1H, m)[a]	4.45(1H, m)
	γ-CH_2			1.4—2.2(2H)	1.5—2.3(2H)	1.8 (1H, m)
						2.1 (1H, m)
	δ-CH_2			3.2 (2H)	3.30(2H, m)	3.32(2H, m)
Tbd	α-CH	5.00(1H, d)	5.01(1H, d)			
	β-CH	4.7 (1H)[a]	4.7 (1H)[a]			
	γ-CH_2	1.4—2.2(2H)	1.5—2.2(2H)			
	δ-CH	5.19(1H)	5.19(1H)			

Abbreviations; s: singlet, d: doublet, dd: double-doublet, q: quartet. a) Chemical shifts in D_2O+TFA.

latter amino acid shifted to a lower field possibly due to the decrease of electron density on its α-carbon atom.

Tuberactinomycins A and B are only distinct from N and O in terms of replacement of capreomycidine with tuberactidine. Only this difference is reflected on their spectra so that a dull singlet attributed to methine proton of δ-carbon atom of Tbd at δ 5.19 appeared in place of the corresponding δ-methylene protons of Cpd at about δ 3.3. The other minor change recognized in spectra was a slightly lower shift of β-methine proton of Tbd compared to that of Cpd owing to the substitution of hydroxyl group at δ-carbon atom. Thus, all protons attached to carbon atom in four tuberactinomycins were successfully assigned as shown in Table 1.

Next, the amide proton resonances in water at pH 2.5 were studied to obtain the informations on a backbone stereochemistry of tuberactinamine and tuberactinomycins in solutions. (Figs. 4 and 5). Tuberactinamine N in H_2O solution at 30 °C (Fig. 4) shows a triplet at δ 8.3 which could be assigned to β-NH of Dpr from its coupling mode. A doublet at δ 9.4 has the same coupling constant (J=12 Hz) to that of a doublet at δ 8.0, which is ascribed to olefinic proton of Uda, and an irradiation on one of the either peaks caused the partner to collapse to a singlet. Therefore, the lower peak was determined to be an amide proton of the ureido group attached to olefinic carbon atom. A singlet at δ 8.95 was assigned to α-NH of Uda from its chemical shift. A highest doublet at δ 7.75 (J=10 Hz) at 30 °C moved to δ 7.68 at 60 °C which was successfully decoupled by an irradiation of a peak centered at δ 4.95 (dd, J=4, 10 Hz) of α-CH of Cpd on a shoulder of H_2O signal. Therefore, the signal of δ 7.75 could be determined as α-NH of Cpd. Two doublets at δ 9.5 and δ 8.8 belong to α-amide protons of two serine residues. The lower doublet was assigned to α-NH of Ser(2) and the higher one to that of Ser(1). The three single peaks corresponding to each two protons at δ 7.4, 6.2, and 6.5 were assigned to two ring NH, *exo* NH_2 protons in guanidino group of Cpd, and primary amide protons in ureido group of Uda, respectively. The lowest signal of them was separated to two peaks with a rise in temperature from 30 °C to 60 °C.

In the spectra of tuberactinomycins A, B, N, and O (Fig. 5), α-NH of Dpr newly appeared in the slightly higher field than that of Ser(1). In the case of tuberactinamine N, the protonated α-amino proton resonance of Dpr could not be observed perhaps due to overlapping with that of water. Except this feature, both spectral patterns of tuberactinomycins N and O were substantially identical with that of tuberactinamine N. On the other hand, in the spectra of tuberactinomycins A and B, all

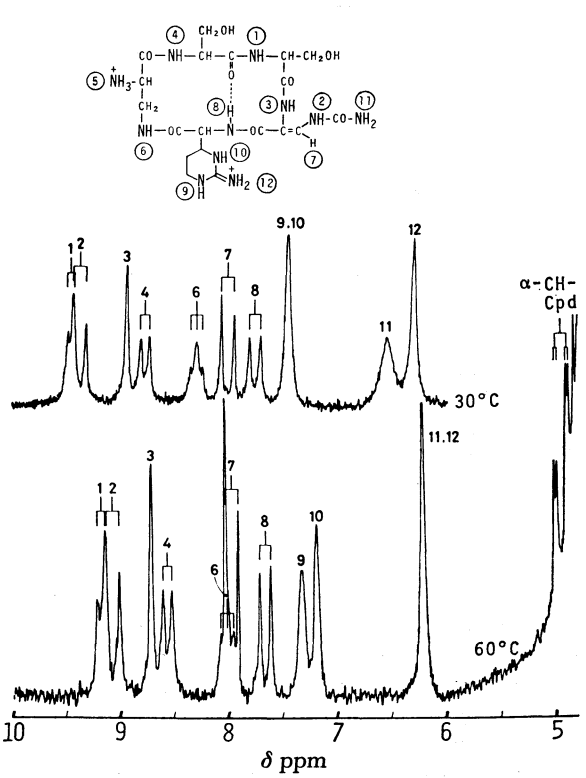


Fig. 4. NMR spectra of tuberactinamine N in low-field region in H_2O at pH 2.5.

of the guanidino proton resonances of Tbd shifted to the lower field compared with those of Cpd in the molecules of another three cyclic peptides. These shifts seem to be apparently effected by the influence of δ-hydroxyl group in Tbd. Especially, δ-NH resonance of Tbd markedly shifted to the downfield and its temperature dependence is relatively small compared with that of β-NH resonance in the same group. This tendency can serve for differentiation of two NH resonances in Cpd ring. Similarly *exo* amino proton as protonated form in Cpd or Tbd was differentiated from a primary amide proton of Uda by a smaller temperature dependence.

In addition to the experiments in H_2O solution, we also studied on spectra in DMSO-d_6 solution. In the spectrum of tuberactinamine N (Fig. 6), the complicated feature in α-methine proton region may be arised from the additional coupling with amide protons. However, the chemical shifts of them were substantially similar to those in D_2O solution. The relation between methine proton and amide proton region was examined by decoupling method on two Ser residues. Thus, an irradiation at the signal of Ser(2) α-CH of δ 4.1 caused the lowest doublet of δ 9.2 to collapse to a singlet. On the other hand, an irradiation at that of Ser(1) α-CH of δ 4.7 forced two doublets at δ 8.6 and 7.5 to collapse to a singlet. From this result, the former doublet can be assigned to that of Ser(1) α-NH, the latter to Cpd α-NH due to the concurrent decoupling with α-CH of Cpd near δ 4.7. The study in DMSO-d_6 solution has consequently supported the assignments of two serine α-NH protons in H_2O solution.

Temperature Dependence. Temperature dependence

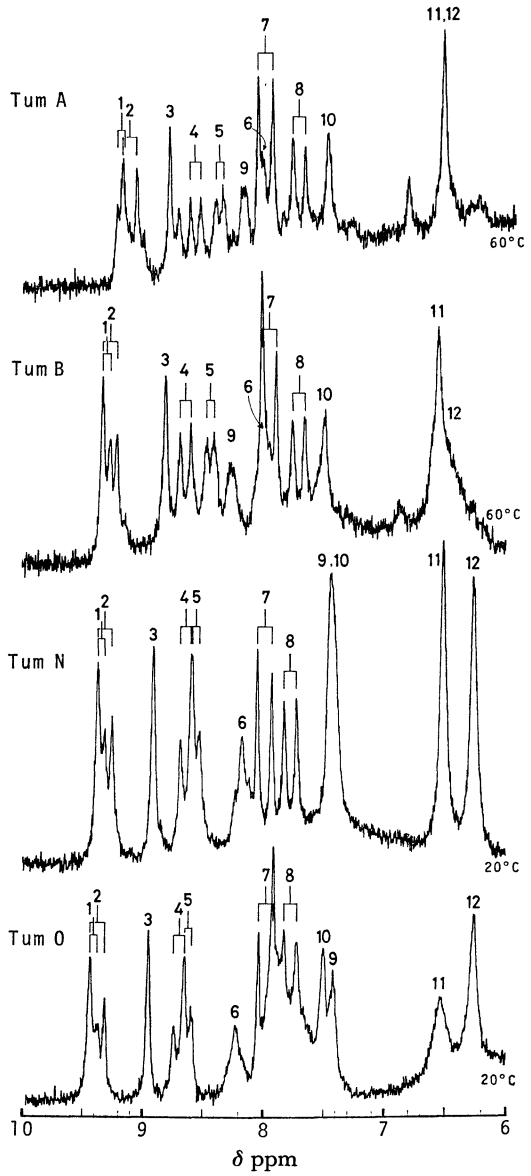


Fig. 5. NMR spectra of tuberactinomycins (Tum) A, B, N, and O in low-field region in H_2O at pH 2.5.

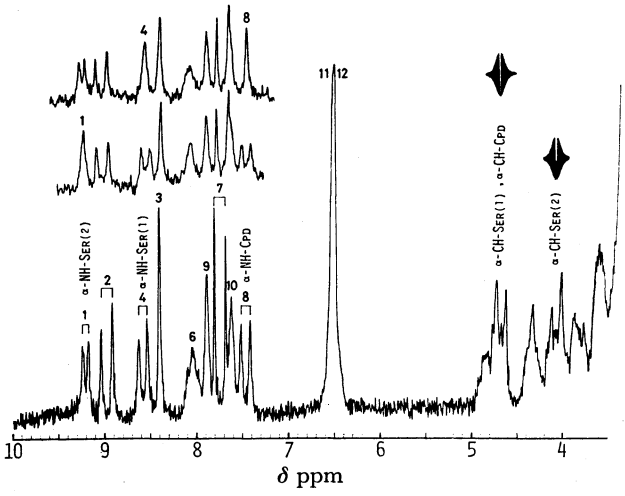


Fig. 6. A part of NMR spectrum of tuberactinamine N in DMSO-d_6.

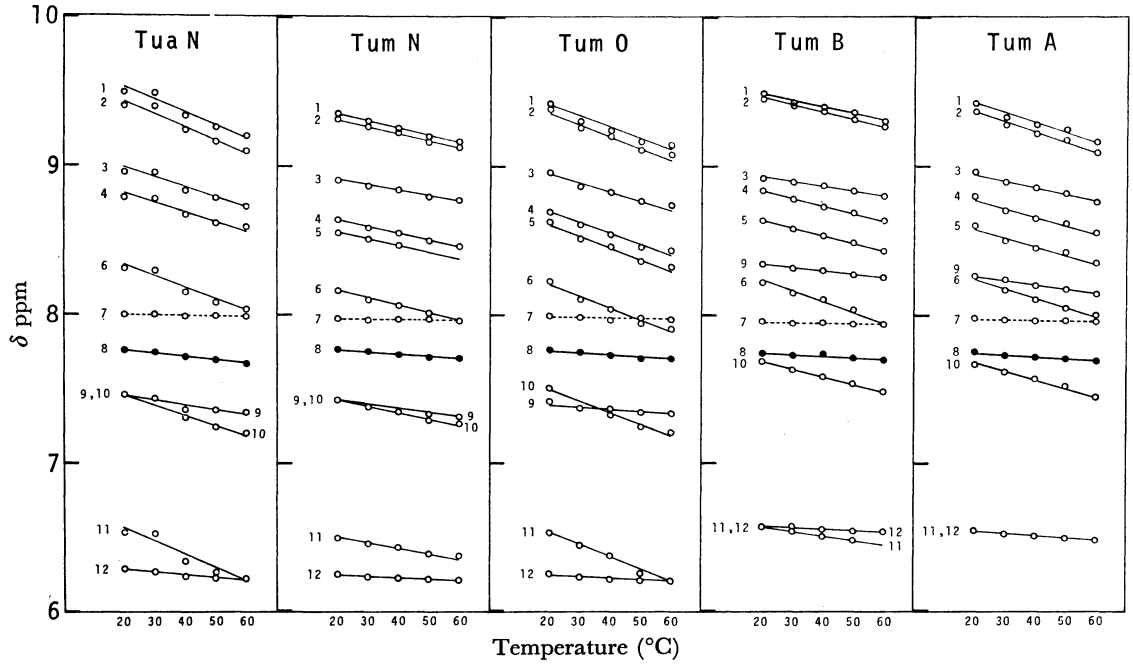


Fig. 7. Temperature dependence of chemical shifts of tuberactinomycins (Tum) and tuberactinamine (Tua) N in low-field region in H_2O at pH 2.5.
○···○ olefinic proton; ●—● α-amide proton of guanidino amino acids; ○—○ other amide and amino protons.

diagram of the lowfield proton resonances in H_2O solution at pH 2.5 of all compounds was given in Fig. 7. Each proton was numbered in order of increasing magnetic field, its assignment being described above. Of these protons, protons **7**, **8**, **9** and **12** showed small temperature coefficients, whereas the other protons shifted markedly to higher field with rise in temperature. Consequently, it appeared that protons **8**, **9**, and **12**, except olefinic proton **7**, were concerned more or less to either intra- or intermolecular hydrogen bond. Especially, the participation of proton **8** corresponding to Cpd or Tbd α-NH is important for comparison of the ring conformations in solutions and crystals. It should be noted that the similar result was obtained in tuberactinamine N which corresponds to the cyclic moiety of the natural peptides.

Deuterium Exchange. Deuterium exchange rates of NH resonances of tuberactinamine N in DMSO-d_6 containing 5% D_2O by volume were studied. Within 15 min after addition of D_2O, all of the resonances except protons **7** and **8** disappeared by exchange with deuteriums. Since the olefinic proton **7** can be excluded from the consideration, it is concluded that the resonance **8** corresponding to Cpd α-NH shows particularly slow exchange rate among NH protons. This proton was not replaced completely by deuteron after 2 hr and its signal remained to some extent even after allowing to stand overnight. The similar tendencies of the corresponding resonances were observed in each spectrum of four tuberactinomycins.

Consequently, it was revealed that only the α-amide proton **8** of Cpd and Tbd formed a fairly protected hydrogen bond in all compounds, while the two protons **9** and **12** may be located in somewhat crowded environment though not participating in the definite hydrogen bond. A carbonyl group as the partner to α-amide proton **8** could not be clarified unambiguously from only the above experiments. However, an intramolecular hydrogen bond was ascertained in crystals of tuberactinomycins O and B (viomycin) between the amide proton of guanidino amino acids and the carbonyl group of Ser(1) as mentioned before. In addition to this fact, taking into account a general information that many cyclic peptides form a stable β-turn structure involving the intramolecular hydrogen bond of ten membered ring, Ser(1) residue is unequivocally chosen as carbonyl partner in these cases. Furthermore the participation of Ser(1) residue in hydrogen bond made possible to understand the significant difference between the resonances of α-methine protons in two serine residues. Thus, the electron density on carbon atom of Ser(1) CO is forced to be lower by the formation of the hydrogen bond. Thereby the protons on atoms linked to Ser(1) CO, *i.e.*, Ser(1) α-CH and Ser(2) α-NH, have to show diamagnetic shift to the lower field.

The backbone conformation of cyclic moiety in crystals of tuberactinomycin O was shown in Fig. 8. The ring part in the molecule was fixed in a definite conformation with the intramolecular hydrogen bond. On the assumption that the conformation in D_2O or H_2O is the same in principle to that in crystals, one can elucidate all the observations consistently. Furthermore, the rigid conformation of tuberactinomycin O in crystals afforded useful informations for side chains in two serine residues. Thus, β-methylene group of Ser(1) was situated in the lateral direction to a nearly lined backbone chain "wall," and thereby the side chain is possible to rotate freely. On the other hand, the β-

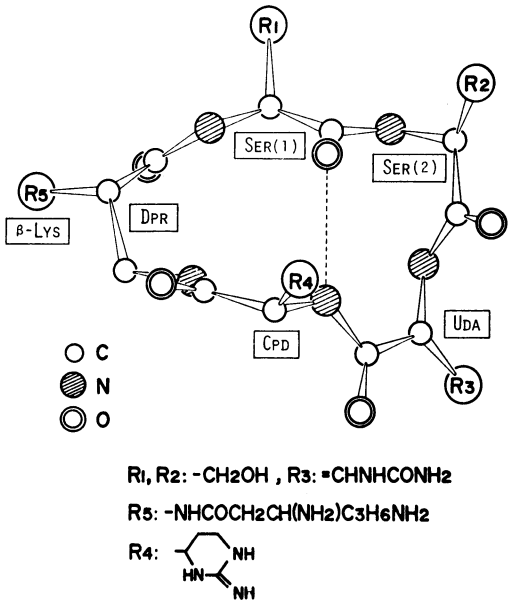


Fig. 8. Backbone conformation of tuberactinomycin O in crystals.

methylene group of Ser(2) was located just on the upper position to the "wall" and consequently a free rotation must be prevented by steric interaction with its own carbonyl group. These magnetically different environments of both side chains made a discrimination between coupling patterns of α- and β-protons. β-Methylene protons in Ser(1) must be coupled to doublet and α-methine proton to triplet, while the both corresponding protons in Ser(2) should appear as double-doublet of ABX type as in fact. The result of this consideration seems to be consistent with the supposition that the conformations of tuberactinamine and tuberactinomycins in solution are almost the same to that in crystals. Furthermore, this conclusion was supported by the following two facts: 1) there are little changes recognized in all α-CH–NH coupling constants at several temperatures in all compounds tested: 2) most of the dihedral angles in the backbone skeleton calculated from NMR spectra in tuberactinomycin O are substantially comparable to those from the result by X-ray analysis.

Recently, Viglino *et al.* reported a NMR study of viomycin, *i.e.*, tuberactinomycin B itself.[14] However, their assignments for some NH resonances did not agree with our results. According to them the lowest signal of singlet mode in acid solution was assigned to Uda α-NH resonance from the comparison of its chemical shift with that of conjugated ureide NH, *i.e.*, –C=CH–NHCONH$_2$. Moreover, another singlet corresponding to our Uda α-NH in tuberactinomycins had been deduced to *exo* imino proton of Tbd in their paper. The guanidino moiety in acid solution must be regarded as protonated form $\overset{-NH}{-NH}\!\!>\!\overset{+}{C}\!-NH_2$ but not as $\overset{-NH}{-NH}\!\!>\!C=$ NH. Consequently, their assignment of Ser(2) α-NH in viomycin was also inconsistent with our result for tuberactinomycins.

From our studies on NMR spectra of tuberactinomycins as mentioned above, some important conclusions were drawn: 1) a strong intramolecular hydrogen bond may serve to maintain a stable and fixed conformation in ring moiety based on β-turn structure in solutions as well as in crystals; 2) such a stable and rigid conformation is common to each antibiotics in tuberactinomycin family; 3) a cyclic peptide moiety, tuberactinamine N, possesses the same conformation with the intramolecular hydrogen bond as those in tuberactinomycin molecules. These results concerning the specific conformation may not be unrelated to the exhibition of antibiotic activities since all of five cyclic peptides showed the comparable antitubercular activities. For the purpose of further investigation on the relationship between conformations of tuberactinomycins and their biological activities, studies on hydrogenated derivatives or synthetic analogues of tuberactinomycins are now in progress.

The authors are deeply indebted to Professor Yoshimasa Kyogoku, Institute for Protein Research, Osaka University, for invaluable discussions. We also wish to express our appreciations to The Research Laboratories, Toyo Jozo Co., Ltd. for the supply of the samples of tuberactinomycins.

References

1) Part VIII. T. Wakamiya and T. Shiba, *J. Antibiot.*, **28**, 292 (1975).
2) Presented at the 12th Symposium on Peptide Chemistry, Kyoto, 1974, p. 135.
3) T. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Take, and J. Abe, *Tetrahedron Lett.*, **1970**, 3497.
4) H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, T. Wakamiya, T. Shiba, and T. Kaneko, *ibid.*, **1971**, 2043.
5) T. Noda, T. Take, A. Nagata, T. Wakamiya, and T. Shiba, *J. Antibiot.*, **25**, 427 (1972).
6) T. Wakamiya, T. Shiba, and T. Kaneko, This Bulletin, **45**, 3668 (1972).
7) T. Wakamiya, T. Shiba, T. Kaneko, H. Sakakibara, T. Noda, and T. Take, *ibid.*, **46**, 949 (1973).
8) T. Wakamiya and T. Shiba, *J. Antibiot.*, **27**, 900 (1974).
9) B. W. Bycroft, *Chem. Commun.*, **1972**, 660.
10) a) A. Stern, W. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci. U. S.*, **61**, 734 (1968); b) F. Conti, *Nature* (London), **221**, 777 (1969); c) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969); d) T. P. Pitner and D. W. Urry, *J. Amer. Chem. Soc.*, **94**, 1399 (1972).
11) a) L. F. Johnson, I. L. Schwartz, and R. Walter, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1269 (1969); b) D. W. Urry and R. Walter, *ibid.*, **68**, 956 (1971); c) R. Walter, R. T. Havran, I. L. Schwartz, and L. F. Johnson, "Proc. Tenth Eur. Peptide Symp., 1969," ed. by E. Scoffone, North-Holland Publ. Co., Amsterdam, (1971), P. 255; d) J. D. Glickson, D. W. Urry, and R. Walter, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2566 (1972); e) R. Walter and J. D. Glickson, *ibid.*, **70**, 1199 (1973).
12) R. Walter, J. D. Glickson, I. L. Schwartz, R. T. Havran, J. Meienhofer, and D. W. Urry, *ibid.*, **69**, 1920 (1972).
13) T. P. Pitner and D. W. Urry, *Biochemistry*, **11**, 4132 (1972).
14) P. Viglino, C. Franconi, A. Lai, E. Brosio, and F. Conti, *Org. Magnetic Resonance*, **4**, 237 (1972).