CHEMICAL STUDIES ON TUBERACTINOMYCIN. XIII[1] MODIFICATION OF β-UREIDODEHYDROALANINE RESIDUE IN TUBERACTINOMYCIN N

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In our previous study[2] on the isolation of the cyclic peptide moiety, tuberactinamine N, from the antibiotic tuberactinomycin N (Fig. 1) through lactonization of the γ-hydroxy-β-lysine residue in the branched part, it was observed that the presence of excess urea on acid treatment of tuberactinomycin N prevented the decomposition of tuberactinamine N, which in the absence of excess urea apparently degraded to the desureido derivative. This decomposition may be attributed to an equilibrium in acidic condition (Fig. 2) between the β-ureidodehydroalanine structure (**1**) and the C-formyl glycine structure (**2**), liberating urea. This aldehyde **2** may further decompose, actually giving several spots on thin-layer chromatogram (silica gel, phenol - water - 28% ammonia, 30: 10: 1). Presence of a large amount of urea seemed to displace the equilibrium to the left-hand side (Fig. 2), resulting in stabilization of the ureido form (**1**). This consideration suggested to us the possibility of a new series of reactions which could lead to a semisynthesis of tuberactinomycin analogs with various structures at the ureido moiety.

Thus, when tuberactinomycin N is treated with acid in the presence of different kinds of urea derivatives, urea exchange could be expected. Accordingly, we attempted to prepare (Fig. 3) N-methylureido (**3**), N,N-dimethylureido (**4**), and thioureido (**5**) analogs of tuberactinomycin N to investigate the role of the unique ureido group in the biological activity of the antibiotic.

In a preliminary experiment to examine an adequate acidity of the medium for the conversion, tuberactinomycin N (20 mg) was first treated with various concentrations of hydrochloric acid (0.5 ml) in the presence of N-methylurea (40 mg) at room temperature. Progress of the reaction was followed by thin-layer chromatography on silica gel using phenol - water - 28% ammonia (30: 10: 1) as developing solvent. In less than 1 N hydrochloric acid, tuberactinomycin N practically failed to undergo the expected urea exchange reaction, whereas in 6~12 N hydrochloric acid, liberation of γ-hydroxy-β-lysine took place to a considerable extent after a few weeks. On the other hand, in 3 N hydrochloric acid, the original spot of tuberactinomycin N was replaced by a new single spot on thin-layer chromatogram after a month. The product isolated as described below retained the γ-hydroxy-β-lysyl moiety and the N-methylureido group as shown by the NMR spectrum (Table 1).

In a standard procedure for the preparation of the ureido analogs, tuberactinomycin N trihydrochloride (1.00 g) was allowed to stand with a given urea derivative (N-methylurea, N,N-dimethylurea or thiourea) (4.00 g) in 3 N hydrochloric acid (10 ml) for one month at 20°C. The reaction mixture was concentrated *in vacuo*, and ethanol was added to the residue to precipitate the crude product. This was then purified

Fig. 1. The structures of tuberactinomycin N and tuberactinamine N

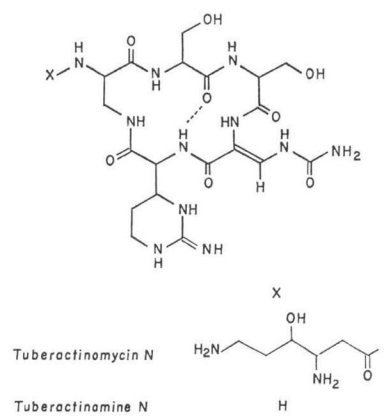


Fig. 2. Equilibrium in acidic solution of β-ureidodehydroalanine residue

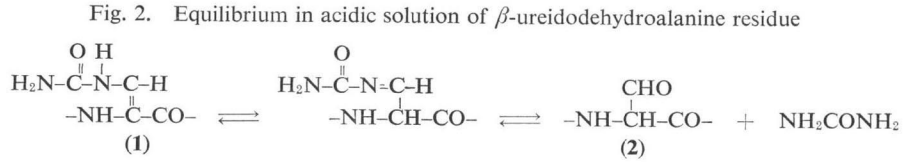
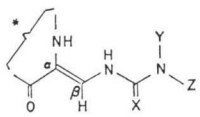


Fig. 3. The structures of [modified β-ureidodehydroalanine[5]]-tuberactinomycins N



	X	Y	Z
3	O	H	Me
4	O	Me	Me
5	S	H	H

* The rest of the molecule is the same as that of tuberactinomycin N.

by column chromatography with Amberlite IRC-50 (Type I, 100~200 mesh, ammonium form, equilibrated with 0.4 M ammonium acetate buffer, pH 9.0), 1.7×30 cm column and eluting with a linear gradient buffer of 0.4 M (350 ml) to 0.8 M ammonium acetate, pH 9.0, (350 ml). Fractions containing the product were neutralized with 3 N hydrochloric acid, diluted with water and then desalted by a column of Amberlite IRC-50 (Type I, 100~200 mesh, pyridinium form, 1.7×

Table 1. The physicochemical properties of the compounds **3**, **4** and **5**

		3 (3HCl)	**4**[a] (3HCl)	**5** (3AcOH)
Yield of conversion (%)		75	64	77
Rf[b]		0.27	0.36	0.22
Rf[c]		0.54	0.52, 0.55	0.61
m.p. (°C, dec.)		232~235	220~222	175~178
$[\alpha]_D^{29}$ (*c* 0.7, H_2O)		−18.7°	−14.0°	−23.2°
Elemental analysis[d] (found (calcd.) (%))	C	38.25 (38.59)	36.44 (36.27)	40.00 (39.78)
	H	6.03 (5.98)	6.80 (6.74)	6.75 (6.57)
	N	22.43 (22.51)	19.28 (19.64)	19.36 (19.46)
		Cl; 13.46 (13.15)	Cl; 11.63 (11.47)	S; 3.35 (3.43)
UV λ_{max} (nm) (ε)	0.1 N HCl	272 (24,900)	275 (25,600)	297 (21,600) 257 (16,700)
	H_2O	272 (24,800)	275 (25,300)	297 (22,000) 257 (17,100)
	0.1 N NaOH	288 (14,000)	290 (16,700)	335 (23,200) 264 (8,940)
NMR[e] (100 MHz) (δ from DSS) (ppm)	in D_2O			
	NCH_3	2.78 (s)	—	—
	$N(CH_3)_2$	—	2.97(s), 3.00(s)	—
	C=CH	8.04(s)	7.40(s), 8.02(s)[g]	8.47(s)
	in H_2O[f]			
	$NHCH_3$	6.65(br.s)	—	—
	$CSNH_2$	—	—	7.7(br.s)
	C=CH	7.98(d,J=12)	7.40(d,J=11) 7.98(d,J=12)	8.46(d,J=12)
	α-NH	8.79(s)	8.83(s), 8.90(s)	8.93(s)
	β-NH	9.15(d,J=12)	8.24(d,J=12) 10.67(d,J=11)	10.04(d,J=12)

a) A mixture of E- and Z-isomers.
b) Tlc (Silica gel, phenol - water - 28% ammonia, 30: 10: 1)
c) Tlc (Silica gel, 10% $AcONH_4$ - acetone - 10% ammonia, 9: 10: 1).
d) The samples for analysis were prepared by reprecipitation from water and methanol.
3: calcd. for $C_{26}H_{45}N_{13}O_{10}\cdot 3HCl$, **4**: calcd. for $C_{27}H_{47}N_{13}O_{10}\cdot 3HCl\cdot 4H_2O\cdot MeOH$,
5: calcd. for $C_{25}H_{43}N_{13}O_9S\cdot 3AcOH\cdot 3H_2O$.
e) Only signals arising from the modified β-ureidodehydroalanine residue were given. The other signals were almost superimposable with those of tuberactinomycin N.
f) In low-field region.
g) Ratio of intensities of the signals was 3: 2 (δ 7.40: E-isomer, δ 8.02: Z-isomer).

30 cm) eluting with 1 N acetic acid. The eluate with positive ninhydrin-reaction was neutralized with pyridine and concentrated *in vacuo*. The desired modified tuberactinomycin N triacetate was precipitated by ethanol and ether.

The physicochemical data on the tuberactinomycin N analogs thus obtained are given in Table 1. All protons except those in the modified β-ureidodehydroalanine part of these three compounds showed the same patterns in their NMR spectra as did those of the natural tuberactinomycin N[3]. Therefore, these analogs and the original antibiotic have similar conformation in solution.

Concerning the configuration of the double bond, N-methylureido derivative (**3**) was shown to have the Z-configuration as does the natural compound since the chemical shift of the olefin proton was observed at δ 8.04 comparable to δ 7.99 of the natural tuberactinomycin N. The thioureido derivative (**5**) seemed to have the same configuration, although in this case a shift to lower field (δ 8.47) was recognized, probably due to the effect of the sulfur atom. The newly introduced double bond in these conversions was forced to the natural configuration presumably due to a conformational effect of the residual moiety of the molecules as was observed during the total synthesis of tuberactinomycin O[4] or its analogs[1]. On the other hand, the N,N-dimethylureido analog (**4**) gave two olefin signals at δ 7.40 and 8.02 indicating that both E- and Z-isomers were formed simultaneously. It is noteworthy that the corresponding two signals (δ 8.24, 10.67) of the β-amide protons (C=C-NH-) in the ureido groups of both isomers deviate appreciably from that of the natural tuberactinomycin N (δ 9.23). This could imply a change of the dihedral angle either of the $C^{\alpha}C^{\beta}$-NC or C^{β}N-CN bond (Fig. 3) in **4** from that of the natural type (180°)[5].

The biological activities of these analogs are shown in Table 2. From these results, it could be concluded that replacement of the hydrogen atom at the terminal amide group in the ureido moiety by methyl group (**3**) has almost no influence on the antibacterial activity of the parent tuberactinomycin N, while introduction of two methyl groups to the terminal amide group (**4**) results in a small but significant lowering of the biological activity, accompanying the scramble of the olefin configuration in the β-ureidodehydroalanine residue and the conformational change of the ureido moiety as discussed above. However, exchange of the ureido with a thioureido group (**5**) did not give any significant change in antibacterial activities.

Table 2. The antimicrobial spectra of the compounds **3**, **4** and **5**, and tuberactinomycin N

Test organisms	MIC (mcg/ml)			
	3	**4**	**5**	Tum N*
Staphylococcus aureus MS353	>100	>100	100	>100
Staphylococcus aureus MS353 AO	>100	>100	100	>100
Streptococcus pyogenes 1022	>100	>100	100	>100
Streptococcus agalactiae 1020	>100	50	>100	>100
Corynebacterium diphtheriae P.W.8	6.3	12.5	3.1	3.1
Bacillus subtilis ATCC 6633	50	100	12.5	25
Escherichia coli NIHJ-JC2	50	>100	50	50
Escherichia coli B	50	>100	50	50
Klebsiella pneumoniae ATCC 10031	25	>100	12.5	25
Salmonella typhosa H 901	50	>100	25	50
Salmonella enteritidis Gaertner	>100	>100	100	100
Shigella flexneri type 3a	>100	>100	100	100
Shigella sonnei E33	100	>100	100	100
Proteus vulgaris OX19	50	>100	50	50
Mycobacterium ATCC 607	12.5	100	12.5	6.3
Mycobacterium 1088	25	>100	25	25

* Tum N: tuberactinomycin N.

Acknowledgement

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References

1) Part XII: TESHIMA, T.; S. NOMOTO, T. WAKAMIYA & T. SHIBA: Chemical studies on tuberactinomycin. XII. Syntheses and antimicrobial activities of [Ala^3, Ala^4]-, [Ala^3]- and [Ala^4]-tuberactinomycin O. Bull. Chem. Soc. Jap. in press

2) WAKAMIYA, T. & T. SHIBA: Chemical studies on tuberactinomycin. VIII. Isolation of tuberactinamine N, the cyclic peptide moiety of tuberactinomycin N, and conversion of tuberactinomycin N to O. J. Antibiotics 28: 292~297, 1975

3) WAKAMIYA, T. & T. SHIBA: Chemical studies on tuberactinomycin. IX. Nuclear magnetic resonance studies on tuberactinomycins and tuberactinamine N. Bull. Chem. Soc. Jap. 48: 2502~2508, 1975

4) TESHIMA, T.; S. NOMOTO, T. WAKAMIYA & T. SHIBA: Chemical studies on tuberactinomycins. X. Total synthesis of tuberactinomycin O. Tetrahedron Letters 1976–27: 2343~2346, 1976

5) YOSHIOKA, H.; T. AOKI, H. GOKO, K. NAKATSU, T. NODA, H. SAKAKIBARA, T. TAKE, A. NAGATA, J. ABE, T. WAKAMIYA, T. SHIBA & T. KANEKO: Chemical studies on tuberactinomycin. II. The structure of tuberactinomycin O. Tetrahedron Letters 1971–23: 2043~2046, 1971