# Chemical Studies on Tuberactinomycin. XI.[1] Semisyntheses of Tuberactinomycin Analogs with Various Amino Acids in Branched Part[2]

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Thirty two semisynthetic tuberactinomycins were prepared by introduction of various amino acids to amino group of tuberactinamine N, a cyclic peptide moiety of tuberactinomycin N and O, which was isolated from natural tuberactinomycin N by acid treatment with liberation of γ-hydroxy-β-lysine of the branched part. Among introduced amino acids, β-amino acids were synthesized from corresponding α-amino acids by modified Arndt-Eistert reaction. After couplings of N-protected amino acids with tuberactinamine N, deprotections were carried out to give semisynthetic tuberactinomycins. From their minimum inhibitory concentrations against many bacteria, it was suggested that a branched part effects significantly to the strength of antimicrobial activities though most important active site must locate in the cyclic peptide moiety, and basicity and/or hydrophobicity of the branched part seemed to strengthen the antibacterial activity especially.

In our recent studies on the antibiotics tuberactinomycins,[3] tuberactinamine N, the cyclic peptide moiety of tuberactinomycin N as well as O, could be isolated as fine crystals by acid treatment of tuberactinomycin N with liberation of γ-hydroxy-β-lysine of the branched part[4] (Fig. 1). Tuberactinamine N thus obtained showed a comparable antituberculous activity to that of original tuberactinomycin N, maintaining the intramolecular hydrogen bond between α-amide proton of capreomycidine residue and carbonyl group of serine[3] residue as in all of tuberactinomycins.[5] From these facts, tuberactinamine N seemed to be a very promising key-intermediate for preparation of novel semisynthetic tuberactinomycins, which have more favorable properties, *e.g.*, high biological activities, less toxicities, and different antibacterial spectra. Such study may also afford very valuable informations concerning relationships between structure and antibacterial activity in tuberactinomycins. Although the cyclic peptide part, *i.e.*, tuberactinamine N, may be the most important site for exhibition of biological activities in tuberactinomycins, its activity is weakened as compared with tuberactinomycin N. This may indicate that the branched amino acid as β-lysine or γ-hydroxy-β-lysine in original tuberactinomycins is required to strengthen the microbial activities.

Along this line, various amino acids were attempted to be linked to the free α-amino group of α,β-diaminopropionic acid residue in tuberactinamine N in this investigation. A number of protected amino acids with benzyloxycarbonyl or *t*-butoxycarbonyl group[6] were coupled with tuberactinamine N by active ester method and then the protecting groups were removed by catalytic hydrogenation or acid treatment respectively (Fig. 2). The second functional groups of α-amino acids like aspartic acid, glutamic acid, serine, histidine, and arginine were protected with *t*-butyl, benzyl, *t*-butyl, tosyl, and nitro groups, respectively, which were removed later together with α-amino-protecting groups. β-Amino acids as materials were prepared from the corresponding α-amino acids by the modified Arndt-Eistert reaction.[7] Furthermore, in this study, we introduced a new convenient way for direct preparation of β-(acylamino) acid *N*-hydroxysuccinimide active

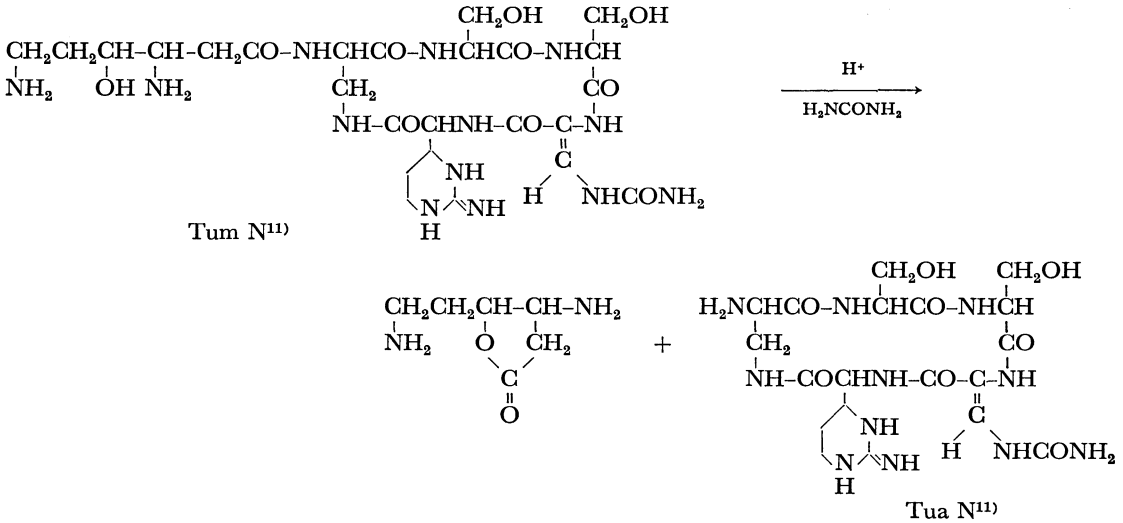


Fig. 1.

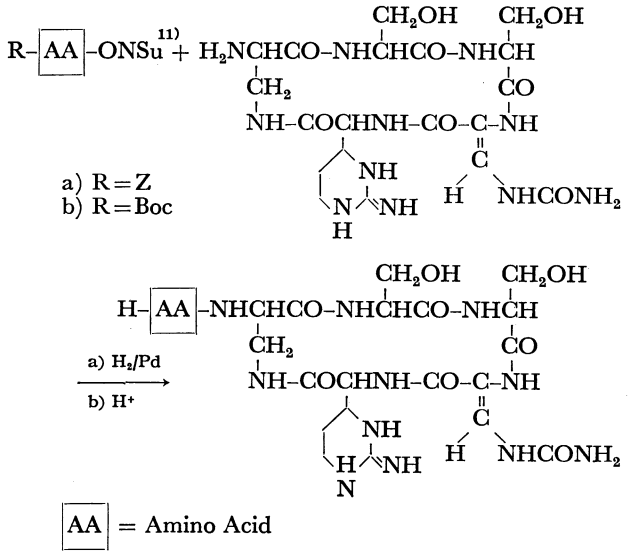


Fig. 2.

ester from diazo ketone derivative of α-(acylamino) acid by use of *N*-hydroxysuccinimide instead of methanol at the step of Wolff rearrangement (Fig. 3).

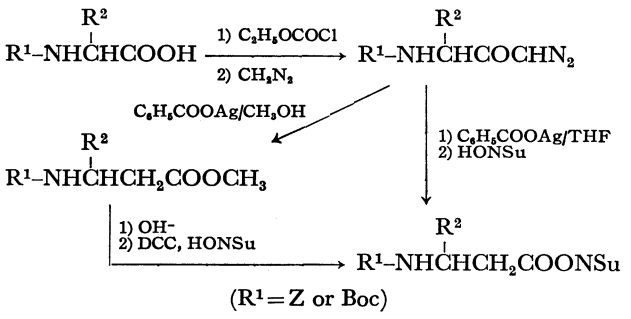


Fig. 3.

Many semisynthetic tuberactinomycins thus prepared were measured their minimum inhibitory concentration (MIC) to various bacilli and studied their ORD and CD spectra.

## Experimental

All melting points are uncorrected. The specific rotations were obtained with a Perkin-Elmer 141 Polarimeter. ORD and CD spectra were obtained with a JASCO Model ORD/UV-5 in water. Thin-layer chromatography was carried out by the ascending method on silica gel G using a developing solvent of butanol–acetic acid–water (4: 1: 2).

*Z-L-$A_2pr$(Z)-ONSu.*[11] To a solution of Z-L-$A_2$pr(Z)-OH (372 mg, 1.0 mmol) in 5 ml of THF, DCC (227 mg, 1.1 mmol) and HONSu (127 mg, 1.1 mmol) were added with stirring at 0 °C. Stirring was continued at this temperature for 1 h and then at room temperature for 6 h. *N,N'*-Dicyclohexylurea was filtered off and filtrate was concentrated *in vacuo*. When crystalline residue was dissolved in ethyl acetate and stored in refrigerator, *N,N'*-dicyclohexylurea was again precipitated. After removal of the precipitate by filtration, hexane was added to the filtrate to obtain fine needles, yield 390 mg (83%), mp 113—116 °C, $[\alpha]_D^{24}$ −36.2° (*c* 1.00, DMF).

Found: C, 58.93; H, 4.99; N, 8.96%. Calcd for $C_{23}H_{23}O_8N_3$: C, 58.84; H, 4.94; N, 8.95%.

*Boc-L-$A_2bu$(Boc)-ONSu.* Boc-L-$A_2$bu(Boc)-OH (oily; 3.18 g, 10.0 mmol), DCC (2.06 g, 10.0 mmol) and HONSu (1.15 g, 10.0 mmol) were treated as described in the preparation of Z-L-$A_2$pr(Z)-ONSu. The product was recrystallized from dioxane and hexane, yield 3.50 g (84%), mp 141—142 °C, $[\alpha]_D^{26}$ −34.4 ° (*c* 1.00, DMF).

Found: C, 52.04; H, 7.03; N, 10.13%. Calcd for $C_{18}H_{29}O_8N_3$: C, 52.04; H, 7.04; N, 10.12%.

*Preparation of β-(Acylamino) Acids* N-*Hydroxysuccinimide Active Ester. Method A (Preparation of Boc-L-β-Lys(Z)-ONSu)*: To a solution of Boc-L-Orn(Z)-OH (3.66 g, 10.0 mmol) in 30 ml of ethyl acetate, *N*-methylmorpholine (1.01 g, 10.0 mmol) and then ethyl chloroformate (1.08 g, 10.0 mmol) were added under cooling in an ice-salt bath and then diazomethane in ether was added. The reaction mixture was stirred for 2 h and allowed to stand at room temperature overnight. A small amount of precipitate was filtered off and filtrate was concentrated *in vacuo*. The yellowish crystalline residue was dissolved in 50 ml of methanol and silver benzoate (100 mg, 0.436 mmol) in 1 ml of triethylamine was added. The reaction mixture was stirred at room temperature for 4 h in the dark, and then concentrated *in vacuo*. Insoluble material of the residue in ethyl acetate was filtered off. Filtrate was washed with 1M hydrochloric acid or 10% citric acid, water, saturated sodium hydrogencarbonate solution, and water to neutral successively. Organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Crystalline residue (3.20 g, 81%) was recrystallized from ethyl acetate and hexane to give a fine Boc-L-β-Lys(Z)-OMe, yield 2.38 g (60%), mp 69—71 °C, $[\alpha]_D^{14}$ −5.1 ° (*c* 1.00, DMF).

Found: C, 60.86; H, 7.74; N, 7.12%. Calcd for $C_{20}H_{30}O_6N_2$: C, 60.89; H, 7.67; N, 7.10%.

To a solution of Boc-L-β-Lys(Z)-OMe (960 mg, 2.43 mmol) in 5 ml of dioxane, 2.43 ml of 1 M sodium hydroxide was added with stirring at 0 °C. Stirring was continued at this temperature for 30 min, and then at room temperature for additional 2 h. The reaction mixture was acidified with 10% citric acid and extracted with ethyl acetate. Organic layer was washed with water to neutral, and was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Crystalline residue thus obtained was recrystallized from ethyl acetate and hexane to give Boc-β-L-Lys(Z)-OH, yield 740 mg (80%), mp 89—91 °C, $[\alpha]_{365}^{14}$ +4.5 ° (*c* 1.01, DMF).

Found: C, 59.98; H, 7.45; N, 7.30%. Calcd for $C_{19}H_{28}O_6N_2$: C, 59.98; H, 7.42; N, 7.36%.

Boc-L-β-Lys(Z)-OH (1.90 g, 5.00 mmol), DCC (1.24 g, 6.00 mmol) and HONSu (690 mg, 6.00 mmol) were allowed to react as described in the preparation of Z-L-$A_2$pr(Z)-ONSu. The product was recrystallized from ethyl acetate and hexane, yield 1.70 g (71%), mp 73 (sintered)—80 °C, $[\alpha]_D^{14}$ −6.1 ° (*c* 1.00, DMF).

Found: C, 57.74; H, 6.56; N, 8.82%. Calcd for $C_{21}H_{31}O_8N_3$: C, 57.85; H, 6.54; N, 8.80%.

*Method B (Preparation of Boc-L-β-Orn(Boc)-ONSu)*: To a solution of Boc-L-$A_2$bu(Boc)-OH (1.59 g, 5.0 mmol) in 25 ml of ethyl acetate, *N*-methylmorpholine (506 mg, 5.0 mmol) and then ethyl chloroformate (543 mg, 5.0 mmol) were added with stirring in an ice-salt bath and stirring was continued for 3 h. *N*-Methylmorpholine hydrochloride was filtered off. A large excess of diazomethane in ether was added to the cold filtrate. A solution was stirred in the cold for 1 h and then at room temperature overnight. Oily residue obtained after vacuum concentration was dissolved in 10 ml of THF. To the solution, HONSu (2.30 g, 20 mmol) and silver benzoate (100 mg, 0.44 mmol) in 1 ml of triethylamine were added. The mixture was stirred at room temperature in the dark for

TABLE 1. PHYSICOCHEMICAL PROPERTIES OF β-(ACYLAMINO) ACID *N*-HYDROXYSUCCINIMIDE ACTIVE ESTERS

| $R^1$-NHCHCH$_2$COONSu ($R^2$) | Method | Yield (%)[a] | Mp (°C) | $[\alpha]_D^{23}$ (*c* 1, DMF) |
|---|---|---|---|---|
| $R^1$=Z, $R^2$=CH(CH$_3$)$_2$ (β-Leu) | B | 79 | 74—76 | +28.5° |
| $R^1$=Z, $R^2$=CH$_2$CH(CH$_3$)$_2$ (β-Hle) | B | 80 | 83—84 | −4.9° |
| $R^1$=Z, $R^2$=CH(CH$_3$)CH$_2$CH$_3$ (β-Hil) | B | 74 | 78—80 | +25.6° |
| $R^1$=Z, $R^2$=CH$_2$C$_6$H$_5$ (β-Hph) | B | 84 | 127—128 | −12.4° |
| $R^1$=Z, $R^2$=CH$_2$CH$_2$CH$_3$ (β-Ahx)[b] | B | 91 | oil | — |
| $R^1$=Z, $R^2$=CH$_2$NH–Z (β, γ-A$_2$bu) | A | 70 | 80[c] | +5.1° |
| $R^1$=Boc, $R^2$=CH$_2$CH$_2$NH–Boc (β-Orn) | B | 71 | 130—132 | −13.8° |
| $R^1$=Boc, $R^2$=CH$_2$CH$_2$CH$_2$NH–Z (β-Lys) | A | 72 | 101—103 | −6.6° |

a) Overall yield from α-(acylamino) acids. b) Only in this case, DL-form of the starting α-amino acid was used while the other starting amino acids were all of L-forms. c) Sintered at 73 °C.

4 h and then concentrated *in vacuo*. After the residue was triturated with ethyl acetate, insoluble inorganic material was filtered off. Filtrate was washed with 10% citric acid, water, saturated sodium hydrogencarbonate solution, and finally water to neutral. Ethyl acetate layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Oily residue was treated with hexane to be crystallized, yield 1.52 g (71%). Recrystallization from ethyl acetate and hexane afforded pure substance, yield 1.26 g (59%), mp 130—132 °C, $[\alpha]_D^{23}$ −13.8 ° (*c* 0.99, DMF).

Found: C, 52.76; H, 7.27; N, 9.65%. Calcd for $C_{19}H_{13}O_8N_3$: C, 53.13; H, 7.28; N, 9.79%.

β-(Acylamino) acid active esters were mainly prepared by method B and their physicochemical properties were listed in Table 1.

*Preparation of New Tuberactinomycin Analogs.* [L-*Lys*[1]]-*Tum N·3HCl*: To a suspension of Tua N·2HCl[4] (300 mg, 0.49 mmol) in 10 ml of DMF, Boc-L-Lys(Boc)-ONSu (324 mg, 0.73 mmol) and triethylamine (51 mg, 0.50 mmol) were added, and stirred at room temperature overnight. Disappearance of Tua N was checked by TLC. A clear solution was concentrated *in vacuo* and the residue was triturated with dioxane or THF.[8] Gelatinous precipitate was filtered or collected by centrifugation. For a complete removal of unreacted active ester, an aqueous solution of the product is better extracted with ethyl acetate. Concentration of the aqueous layer *in vacuo* gave a pure coupling product in a quantitative yield as hygroscopic powder. It was immediately dissolved in 3 ml of 3 M hydrochloric acid and allowed to stand for 45 min at room temperature. Additions of ethanol and ether gave precipitate, yield 340 mg (89%). It was recrystallized from water and ethanol to give fine needles 307 mg, 81%), mp 236—237 °C (dec), $[\alpha]_D^{24}$ −9.8 ° (*c* 1.00, $H_2O$).

Found: C, 38.36; H, 6.13; N, 23.01; Cl, 13.36%. Calcd for $C_{25}H_{43}O_9N_{13}\cdot 3HCl\cdot 1/2H_2O$: C, 38.10; H, 6.01; N, 23.11; Cl, 13.50%.

[L-β-*Leu*[1]]-*Tum N*: To a suspension of Tua N·2HCl[4] (307 mg, 0.50 mmol) in 10 ml of DMF, Z-L-β-Leu-ONSu (217 mg, 0.60 mmol) and triethylamine (61 mg, 0.60 mmol) were added. After Tua N was consumed, a clear solution was concentrated *in vacuo*. The residue was triturated with THF and gelatinous product was collected by centrifugation. Powder obtained quantitatively was dissolved in water containing 0.1 ml of concentrated hydrochloric acid and extracted with ethyl acetate several times. The aqueous layer was subjected to hydrogenation by use of palladium black catalyst. The reaction was followed by TLC and catalyst was filtered off after completion of debenzyloxycarbonylation. Filtrate was concentrated *in vacuo* and the residue was treated with ethanol to make it powder, yield 350 mg (96%). It was recrystallized from water and ethanol, yield 300 mg (82%), mp 238 °C (dec), $[\alpha]_D^{24}$ −22.7 ° (*c* 1.00, $H_2O$).

Found: C, 40.07; H, 6.16; N, 22.23; Cl, 9.25%. Calcd for $C_{25}H_{42}O_9N_{12}\cdot 2HCl\cdot H_2O$: C, 40.27; H, 6.22; N, 22.54; Cl, 9.51%.

All of the other acids applied were coupled to the Tua N *via* *N*-hydroxysuccinimide ester, in similar manners, by

TABLE 2. PHYSICOCHEMICAL PROPERTIES OF SEMISYNTHETIC TUBERACTINOMYCIN ANALOGS (R-Tua N)

| R | Mp (dec) (°C) | $[\alpha]_D^6$ (*c* 1.0, $H_2O$) | Yield (%) | Protecting group of amino group |
|---|---|---|---|---|
| Asp | 245 | −17.2° | 94 | Z |
| Glu[f] | 246 | −19.2° | 91 | Boc |
| Gly | 232—234 | −20.7° | 63 | Boc |
| Ala | 248—249 | −13.5° | 75 | Z |
| Ser | 235—236 | −16.5° | 66 | Boc |
| Val | 253—254 | −13.6° | 88 | Z |
| Leu | >250 | −15.3° | 88 | Z |
| Ile | 247—248 | −14.9° | 86 | Z |
| Phe | 233—235 | −7.6° | 87 | Z |
| Tyr | 236—238 | −3.8° | 32 | Boc |
| Trp | 248—249 | +3.5° | 53 | Boc |
| Pgl[f],[11] | 247—249 | −9.2° | 48 | Z |
| Pro | 235 | −37.4° | 73 | Z |
| β-Ala | 232—234 | −23.2°[a] | 91 | Z |
| β-Leu | 238 | −22.7° | 82 | Z |
| β-Hle | 239—240 | −19.6° | 83 | Z |
| β-Hil | 240—241 | −14.8° | 78 | Z |
| β-Hph | 241 | −19.0° | 88 | Z |
| β-Ahx | 244—245 | −20.1° | 69 | Z |
| ε-Ahx | 233—234 | −25.6° | 90 | Boc |
| Lys | 236—237 | −9.8°[c] | 81 | Boc |
| Lys[f] | 236—238 | −28.8° | 30 | Boc |
| Orn | 241—243 | −9.0°[a] | 83 | Boc |
| α, γ-A$_2$bu | 234—235 | −2.0° | 79 | Boc |
| α, β-A$_2$pr | >250 | −4.3° | 77 | Z |
| β-Orn | 241—243 | −14.0° | 83 | Boc |
| β, γ-A$_2$bu | 249 | −14.1° | 85 | Z |
| Arg | >250 | −8.7°[b] | 53 | Nps |
| His | 229—231 | −3.9° | 53 | Boc |
| Ac-β-Lys | 239 | −34.0°[e] | 55 | Z |
| β-Lys(Ac) | 236 | −24.2°[e] | 65 | Boc |
| Ac-β-Lys(Ac) | 215 | −34.6°[d] | 68 | — |

a) at 11 °C. b) at 16 °C. c) at 24 °C. d) at 25 °C. f) D-from.

either procedure mentioned above depending on the protecting group used except in the case of arginine. All protecting groups in the products were finally removed with acid or by hydrogenation in the presence of acid. However $N^{im}$-tosyl group of histidine was readily removed with HOBt after coupling reaction.[9] Physicochemical data for all other semisynthetic tuberactinomycins are listed in Table 2.

*[L-Arg¹]-Tum N·3HCl.* To a solution of Tua N·2HCl[4] (300 mg, 0.49 mmol), Nps–Arg($NO_2$)–OH (181 mg, 0.54 mmol), and HOBt (72 mg, 0.54 mmol) in a mixture of 5 ml of DMF and 4 ml of water, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (78 mg, 0.54 mmol) was added with stirring in an ice bath. It was stirred in an ice bath for 3 h and then at room temperature overnight. The solution thus obtained was concentrated *in vacuo* and the residue was triturated with ethyl acetate. Precipitate was filtered and then suspended in 10 ml of 1 M hydrochloric acid and 10 ml of ether. The mixture was stirred at room temperature for 30 min. Aqueous layer separated was washed with ether three times. The aqueous solution was concentrated *in vacuo* and the residue was reprecipitated from water and ethanol to remove sulfur-containing compound completely. Precipitate was again dissolved in 20 ml of water containing 0.5 ml of concd hydrochloric acid and hydrogenated in the presence of palladium black. After completion of deprotection, the catalyst was removed by filtration. Filtrate was concentrated *in vacuo* and the residue was treated with ethanol to give powder, yield 355 mg (90%). For purification, it was applied to Amberlite IRC 50 (CG 50 Type I 1.5×23 cm) column and eluted gradiently with pH 3.1 to pH 5.0 buffer (0.2—2 M pyridine–acetate). Eluates containing [L-Arg¹]-Tum N were collected and concentrated *in vacuo*. To a solution of the residue in 6 M hydrochloric acid, ethanol was added to precipitate the product, yield 208 mg (53%), mp >250 °C, $[\alpha]_D^{16}$ −8.7 ° (*c* 1.01, $H_2O$).

Found: C, 37.05; H, 5.84; N, 24.86; Cl, 12.76%. Calcd for $C_{25}H_{43}O_9N_{15}\cdot 3HCl\cdot H_2O\cdot 1/2C_2H_5OH$: C, 36.82; H, 6.06; N, 24.77; Cl, 12.54%.

*[Ac–L-β-Lys¹]-Tum N (Nᵝ-Ac–Tum O)·2HCl.* To a suspension of Tua N·2HCl[4] (1.50 g, 2.44 mmol) in 50 ml of DMF, Boc–L-β-Lys(Z)–ONSu (1.28 g, 2.69 mmol) and triethylamine (245 mg, 2.44 mmol) were added. After completion of reaction, a clear solution was concentrated *in vacuo* and oily residue was triturated with THF to make gelatinous precipitate of diacyl peptide, which was collected by centrifugation, yield 1.94 g (89%).

For removal of Boc group, an aliquot of the product (200 mg, 0.22 mmol) was dissolved in a mixture of 50% acetic acid (1 ml) and concd hydrochloric acid (0.5 ml), and then stirred at room temperature for 1.5 h. Addition of ethanol and ether to the solution gave white precipitate, yield 179 mg (92%). To a solution of the product (179 mg, 0.20 mmol) in 10 ml of DMF, AcONSu (35 mg, 0.22 mmol) and triethylamine (23 mg, 0.22 mmol) were added. The mixture was stirred at room temperature overnight and then concentrated *in vacuo*. When oily residue was triturated with THF, gelatinous precipitate was formed which was immediately hydrogenated in aqueous solution containing 0.5 ml of 1 M hydrochloric acid by use of palladium black catalyst. After completion of hydrogenolysis, catalyst was filtered off and filtrate was concentrated *in vacuo*. The residue was treated with ethanol to make it powder, yield 113 mg (71%), which was reprecipitated from water and ethanol to give pure desired material, yield 89 mg (55%), mp 239 °C (dec), $[\alpha]_D^{26}$ −34.0 ° (*c* 1.00, $H_2O$).

Found: C, 40.79; H, 6.12; N, 22.76; Cl, 8.99%. Calcd for $C_{27}H_{47}O_{10}N_3\cdot 2HCl\cdot 1/2H_2O$: C, 40.86; H, 6.10; N, 22.93; Cl, 8.93%.

*[L-β-Lys(Ac)¹]-Tum N (Nᵋ-Ac–Tum O)·2HCl.* [Boc–L-β-Lys(Z)¹]-Tum N (*N*ᵝ-Boc–*N*ᵋ-Z–Tum O)·2HCl (200 mg, 0.22 mmol) obtained during the above procedure was dissolved in DMF and hydrogen was bubbled with stirring in the presence of palladium black. Debenzyloxycarbonylation was completed in 30 h and thereafter catalyst was filtered off. After addition of AcONSu (35 mg, 0.22 mmol) to filtrate, the reaction mixture was stirred at room temperature for 15 h and concentrated *in vacuo*. The residue was treated with THF to give a single product of solid which was then dissolved in 4 M hydrochloric acid to remove Boc group. After 1 h, ethanol was added to the solution precipitating a product. Addition of ether completed the precipitation, yield 143 mg (82%). Product was reprecipitated from water and ethanol to obtain a pure compound, yield 114 mg (65%), mp 236 °C (dec), $[\alpha]_D^{26}$ −24.2 ° (*c* 1.01, $H_2O$).

Found: C, 40.70; H, 6.14; N, 22.66; Cl, 9.00%. Calcd for $C_{27}H_{45}O_{10}N_3\cdot 2HCl\cdot 1/2H_2O$: C, 40.86; H, 6.10; N, 22.93; Cl, 8.93%.

*[Ac–β-Lys(Ac)¹]-Tum N (Nᵝ,Nᵋ-(Ac)₂–Tum O)·HCl.* AcONSu (88 mg, 0.56 mmol) and triethyl amine (53 mg, 0.52 mmol) were added to a suspension of Tum O·3HCl (200 mg, 0.26 mmol) in 10 ml of DMF. The mixture was stirred for 20 h. Gelatinous residue obtained after vacuum concentration was treated with THF to give powder in a quantitative yield. Recrystallization from water and ethanol gave a pure product, yield 139 mg (68%), mp 215 °C (dec), $[\alpha]_D^{25}$ −34.6 ° (*c* 1.02, $H_2O$).

Found: C, 43.28; H, 6.23; N, 22.55; Cl, 4.32%. Calcd for $C_{29}H_{47}O_{11}N_{13}\cdot HCl\cdot H_2O$: C, 43.09; H, 6.24; N, 22.53; Cl, 4.39%.

## Results and Discussions

Minimum inhibitory concentrations of semisynthetic tuberactinomycins preparated in this study to many bacteria were listed in Table 3. Several noticeable features were summarized as follows: 1) Although most of the synthetic compounds showed more or less antimicrobial activity against *Mycobacterium* and *Corynebacterium*, the coupling of basic amino acids gave favorable results in general. 2) Acidic amino acids as branch part extinguish the activity at all. 3) Coupling of neutral amino acids with bulky or hydrophobic side chain was relatively effective particularly against *Mycobacterium* as well as in the case of basic amino acids. 4) Remarkable relationship between methylene lengths in diamino acids and their activities against *Mycobacterium* (Table 4) was recognized. 5) Among semisynthetic tuberactinomycins prepared in this study only [L-β-Orn¹]-Tum N showed a similar antimicrobial spectrum and comparable activity to that of natural Tum N. From above results, it can be inferred that a fairly strict structure is required at a branch amino acid part to maintain a full antimicrobial activity of natural compounds, *i.e.*, presence of β-amino group and of longer chain than three carbons between two amino groups in diamino acid are necessary.

In connection with the last assumption, the role of both amino groups of β-lysine in natural tuberactinomycins was next investigated by the preparation of five following derivatives, *i.e.*, [β-Ahx¹]-Tum N, [ε-Ahx¹]-

TABLE 3. MINIMUM INHIBITORY CONCENTRATIONS[a] OF SEMISYNTHETIC TUBERACTINOMYCIN ANALOGS (R-Tua N)

| Test Organisms[b] | Asp | Glu[c] | Gly | Ala | Ser | Val | Leu | Ile | Phe | Tyr | Trp |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Corynebacterium diphtheriae* P.W. 8 | >100 | >100 | >100 | >100 | >100 | >100 | 25 | >100 | 50 | 100 | 25 |
| *Bacillus subtilis* ATCC 6633 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Escherichia coli* NIHJ | >100 | >100 | >100 | >100 | >100 | >100 | 100 | >100 | >100 | >100 | >100 |
| *Escherichia coli* B | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Salmonella typhosa* H 901 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Shigella sonnei* E33 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Klebsiella pneumonia* ATCC 10031 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Mycobacterium* ATCC 607 | >100 | >100 | 50 | 25 | 100 | 25 | 6.3 | 25 | 50 | 12.5 | 6.3 |

| Test Organisms[b] | Pgl[c] | Pro | β-Ala | β-Leu | β-Hle | β-Hil | β-Hph | β-Ahx[d] | ε-Ahx | Lys | Lys[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Corynebacterium diphtheriae* P.W. 8 | 100 | 100 | 100 | 50 | 50 | 50 | 50 | 100 | 100 | 12.5 | 50 |
| *Bacillus subtilis* ATCC 6633 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 100 | 100 |
| *Escherichia coli* NIHJ | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Escherichia coli* B | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Salmonella typhosa* H 901 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 100 | >100 |
| *Shigella sonnei* E33 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Klebsiella pneumonia* ATCC 10031 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| *Mycobacterium* ATCC 607 | 25 | >100 | 100 | 50 | 100 | 100 | 100 | 100 | >100 | 6.3 | 12.5 |

| Test Organisms[b] | Orn | $A_2$bu | $A_2$pr | β-Orn | β,γ-$A_2$bu | Arg | His | Ac–β-Lys | Ac / β-Lys | Ac / Ac–β-Lys | Tum N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Corynebacterium diphtheriae* P.W. 8 | 50 | 100 | 100 | 12.5 | 100 | — | — | >100 | 100 | >100 | 6.3 |
| *Bacillus subtilis* ATCC 6633 | 100 | >100 | >100 | 25 | >100 | >100 | >100 | >100 | >100 | >100 | 25 |
| *Escherichia coli* NIHJ | >100 | >100 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | 50 |
| *Escherichia coli* B | >100 | >100 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | 50 |
| *Salmonella typhosa* H 901 | >100 | >100 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | 50 |
| *Shigella sonnei* E33 | >100 | >100 | >100 | 50 | >100 | >100 | >100 | >100 | >100 | >100 | 50 |
| *Klebsiella pneumonia* ATCC 10031 | >100 | >100 | >100 | 25 | >100 | >100 | >100 | >100 | >100 | >100 | 50 |
| *Mycobacterium* ATCC 607 | 25 | 50 | 100 | 12.5 | 50 | 25 | >100 | >100 | 100 | >100 | 6.3 |

a) μg/ml. b) All of semisynthetic Tum analogs were inactive to the following organisms: *Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* sp-al-1, *Streptococcus pyogenes* N.Y.5, *Sarcina lutea* ATCC 9341, *Micrococcus flavus* ATCC 10240, *Salmonella paratyphi* PA 41-N-22, *Salmonella enteritidis* Gaertner, *Shigella flexineri* type 3a, *Proteus vulgaris* OX 19, *Serratia marcescens*, *Pseudomonas aeruginosa* IAM 1095. c) D-form. d) DL-form. The other amino acids were of L-form.

TABLE 4. RELATIONSHIP BETWEEN METHYLENE LENGTHS IN DIAMINO ACIDS AND ACTIVITIES AGAINST MYCOBACTERIUM ATCC 607

| α,ω-Diamino acids | MIC (μg/ml)[a] |
|---|---|
| $CH_2CH_2CH_2CH_2CH(NH_2)$–$CH(NH_2)$–CO– | 6.3 (D=12.5) |
| $CH_2CH_2CH_2CH(NH_2)$–$CH(NH_2)$–CO– | 25 |
| $CH_2CH_2CH(NH_2)$–$CH(NH_2)$–CO– | 50 |
| $CH_2CH(NH_2)$–$CH(NH_2)$–CO– | 100 |

| β,ω-Diamino acids | MIC(μg/ml)[a] |
|---|---|
| $CH_2CH_2CH_2CH(NH_2)$–$CH(NH_2)$–$CH_2$CO–[b] | 6.3 |
| $CH_2CH_2CH(NH_2)$–$CH(NH_2)$–$CH_2$CO– | 12.5 |
| $CH_2CH(NH_2)$–$CH(NH_2)$–$CH_2$CO– | 50 |

a) MIC: Minimum inhibitory concentration.
b) Tum O.

Tum N, $N^{\beta}$-Ac–Tum O, $N^{\varepsilon}$-Ac–Tum O, and $N^{\beta},N^{\varepsilon}$-$(Ac)_2$–Tum O.[11] Antimicrobial activities of them were markedly affected by such modifications as can be seen in Table 3. Disappearance of the activities in all derivatives indicated the indispensability of both free amino groups, being consistent with the results mentioned before. From observation that weak activities remain in [β-Ahx[1]]-Tum N and $N^{\varepsilon}$-Ac–Tum O compared to three other derivatives, β-amino group seems to be slightly rather effective than ω-one. Recently, Kitagawa and his collaborators also pointed out the significance of both amino groups in β-lysine residue from their study on viomycin.[10]

In order to elucidate a relationship between the structure and biological activity of those semisynthetic antibiotics prepared here, ORD and CD of several synthetic as well as natural tuberactinomycins were measured. Their spectra were depicted in Fig. 4, in which extreme similarities in patterns are noticed. Thus we could understand that molecules of semisynthetic tuberactinomycins are forced to a definite conformation fixed by an intramolecular hydrogen bond[5] in cyclic peptide moiety despite of large difference in branched

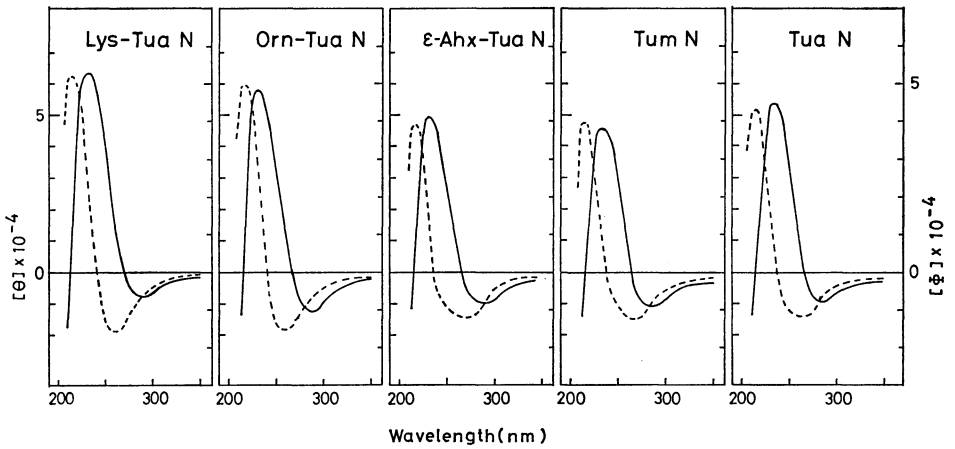


Fig. 4. ORD and CD curves of Tum analogs. ——: ORD, ------: CD, solvent: water.

part structure. Most important conclusion is that such conformation of whole molecule is not necessarily related to the biological activity, since inactive [ε-Ahx[1]]-Tum N showed quite similar pattern of ORD and CD spectra to natural antibiotics.

Activities against human tubercule bacilli, either sensitive or resistant to tuberactinomycin B, were also measured for some of the semisynthetic tuberactinomycins which are active to *Mycobacterium* ATCC 607, as shown in Table 5. While they showed significant antibacterial activity to sensitive human tubercule bacillus, none of them showed antibacterial activity to human tubercule bacillus resistant to tuberactinomycin B. Therefore any changes of the branched amino acid could not effect on cancellation of drug-resistancy of human tubercule bacilli so far.

In conclusion, all the results obtained in the present study can be summarized as follows. Most important active site in antibiotics of tuberactinomycin family must be in the cyclic peptide moiety, though its presence does not assure an exhibition of full biological activity, and basicity and/or hydrophobicity of the molecule seems to be the second important requisite for antimicrobial activity. At present time, there are found no semisynthetic compounds with stronger activity or more favorable quality than natural ones. However this study could explore a new way to modify natural tuberactinomycins by different kinds of acylation for the purpose of searching more desirable analogs.

Table 5. Minimum inhibitory concentrations[a] of semisynthetic tuberactinomycin analogs against mycobacterium and human tubercule bacilli

| | *Mycobacterium* ATCC 607 | *Human tubercule bacillus* | *Human tubercule bacillus* (200μg-Resistance to Tum B) |
|---|---|---|---|
| Leu-Tua N | 6.3 | 25 | >200 |
| Ile-Tua N | 12.5 | 25 | >200 |
| Tyr-Tua N | 12.5 | 25 | >200 |
| β-Orn-Tua N | 12.5 | 25 | >200 |
| Trp-Tua N | 6.3 | 25 | >200 |
| Tum N | 6.3 | 25 | >200 |

a) μg/ml.

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11) Abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. Tum: tuberactinomycin, Tua: tuberactinamine, DCC: dicyclohexylcarbodiimide, HONSu: *N*-hydroxysuccinimide, HOBt: 1-hydroxybenzotriazole, Nps: *o*-nitrophenylsulphenyl, Z: benzyloxycarbonyl, Boc: *t*-butoxycarbonyl, DMF: *N,N*-dimethylformamide, THF: tetrahydrofuran, Pgl: phenylglycine, Hle: homoleucine, Hil: homoisoleucine, Hph: homophenylalanine, Ahx: aminohexanoic acid, $A_2$bu: α,γ-diaminobutyric acid, β,γ-$A_2$bu: β,γ-diaminobutyric acid, $A_2$pr: α,β-diaminopropionic acid.