CHEMICAL STUDIES ON TUBERACTINOMYCIN. XIII\(^{1)}\) MODIFICATION OF \(\beta\)-UREIDODEHYDROALANINE RESIDUE IN TUBERACTINOMYCIN N

S. NOMOTO and T. SHIBA*  
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan  
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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 \(\gamma\)-hydroxy-\(\beta\)-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 \(\beta\)-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 \(\gamma\)-hydroxy-\(\beta\)-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 \(\gamma\)-hydroxy-\(\beta\)-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.

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

![Fig. 1. The structures of tuberactinomycin N and tuberactinamine N](image)

Fig. 2. Equilibrium in acidic solution of \(\beta\)-ureidodehydroalanine residue

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{H}_2\text{N}-\dot{\text{C}}-\text{N}-\text{C}-\text{H} & \quad \text{H}_2\text{N}-\dot{\text{C}}-\text{N}-\text{C}-\text{H} \\
\text{CHO} & \quad \text{H}_2\text{N}-\dot{\text{C}}-\text{N}-\text{C}-\text{H} \\
\text{NH}_2\text{CONH}_2 & \quad \text{NH}_2\text{CONH}_2
\end{align*}
\]
Fig. 3. The structures of [modified β-ureidodehydroalanine]-tuberactinomycins N

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

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield of conversion (%)</th>
<th>Rf b)</th>
<th>Rf c)</th>
<th>m.p. (°C, dec.)</th>
<th>[α]D (c 0.7, H2O)</th>
<th>Elemental analysis</th>
<th>UV λmax (nm)</th>
<th>NMR (100 MHz)</th>
<th>δ from DSS (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (3HCl)</td>
<td>75</td>
<td>0.27</td>
<td>0.54</td>
<td>232 ~ 235</td>
<td>-18.7°</td>
<td>C 38.25 (38.59)</td>
<td>272 (24,900)</td>
<td>8.04(s)</td>
<td>6.65(br.s)</td>
</tr>
<tr>
<td>4° (3HCl)</td>
<td>64</td>
<td>0.36</td>
<td>0.52, 0.55</td>
<td>220 ~ 222</td>
<td>-14.0°</td>
<td>H 6.03 (5.98)</td>
<td>272 (24,800)</td>
<td>7.40(s)</td>
<td>6.65(br.s)</td>
</tr>
<tr>
<td>5 (3AcOH)</td>
<td>77</td>
<td>0.22</td>
<td>0.61</td>
<td>175 ~ 178</td>
<td>-23.2°</td>
<td>N 22.43 (22.51)</td>
<td>288 (14,000)</td>
<td>7.98(d,J=12)</td>
<td>7.98(d,J=12)</td>
</tr>
</tbody>
</table>

a) A mixture of E- and Z-isomers.
b) Tlc (Silica gel, phenol - water - 28% ammonia, 30: 10: 1)
c) Tlc (Silica gel, 10% AcONH4 - acetone - 10% ammonia, 9: 10: 1).
d) The samples for analysis were prepared by reprecipitation from water and methanol.
3: calcd. for C30H45N13O10·3HCl, 4: calcd. for C31H47N13O10·3HCl·4H2O·MeOH,
5: calcd. for C29H43N13O9S·3AcOH·3H2O.
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 \(\delta 8.04\) comparable to \(\delta 7.99\) of the natural tuberactinomycin N. The thioureido derivative (5) seemed to have the same configuration, although in this case a shift to lower field (\(\delta 8.47\)) 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 moeity of the molecules as was observed during the total synthesis of tuberactinomycin O\(^1\) or its analogs\(^2\). On the other hand, the N,N-dimethylureido analog (4) gave two olefin signals at \(\delta 7.40\) and 8.02 indicating that both E- and Z-isomers were formed simultaneously. It is noteworthy that the corresponding two signals (\(\delta 8.24, 10.67\)) of the β-amide protons (C=\(\text{C}-\text{NH}\)-) in the ureido groups of both isomers deviate appreciably from that of the natural tuberactinomycin N (\(\delta 9.23\)). This could imply a change of the dihedral angle either of the C=\(\text{C}\)-NC or C\(\text{N}\)-CN bond (Fig. 3) in 4 from that of the natural type (\(180^\circ\))\(^3\).

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 thio-ureido group (5) did not give any significant change in antibacterial activities.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>MIC (mcg/ml)</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Tum N*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus MS353</em></td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>100</td>
<td>&gt;100</td>
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<tr>
<td><em>Staphylococcus aureus MS353 AO</em></td>
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<td>&gt;100</td>
<td>&gt;100</td>
<td>100</td>
<td>&gt;100</td>
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<tr>
<td><em>Streptococcus pyogenes 1002</em></td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae 1020</em></td>
<td></td>
<td>&gt;100</td>
<td>50</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td><em>Corynebacterium diphtheriae P.W.8</em></td>
<td></td>
<td>6.3</td>
<td>12.5</td>
<td>3.1</td>
<td>3.1</td>
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<tr>
<td><em>Bacillus subtilis ATCC 6633</em></td>
<td></td>
<td>50</td>
<td>100</td>
<td>12.5</td>
<td>25</td>
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<tr>
<td><em>Escherichia coli NIHJ-JC2</em></td>
<td></td>
<td>50</td>
<td>&gt;100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><em>Escherichia coli B</em></td>
<td></td>
<td>50</td>
<td>&gt;100</td>
<td>50</td>
<td>50</td>
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<td><em>Klebsiella pneumoniae ATCC 10031</em></td>
<td></td>
<td>25</td>
<td>&gt;100</td>
<td>12.5</td>
<td>25</td>
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<tr>
<td><em>Salmonella typhosa H 901</em></td>
<td></td>
<td>50</td>
<td>&gt;100</td>
<td>25</td>
<td>50</td>
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<tr>
<td><em>Salmonella enteritidis Gaertner</em></td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>100</td>
<td>100</td>
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<tr>
<td><em>Shigella flexneri type 3a</em></td>
<td></td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td><em>Shigella sonnei E33</em></td>
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<td><em>Proteus vulgaris OX19</em></td>
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<td>6.3</td>
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<td><em>Mycobacterium 1088</em></td>
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<td>25</td>
<td>&gt;100</td>
<td>25</td>
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</table>

* Tum N: tuberactinomycin N.
Acknowledgement

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