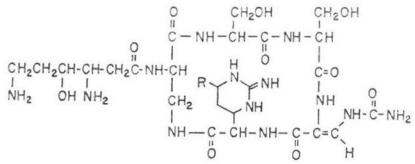
CHEMICAL STUDIES ON TUBERACTINOMYCIN. VI
THE ABSOLUTE CONFIGURATION OF γ-HYDROXY-β-LYSINE IN TUBERACTINOMYCINS A AND N[1]

Sir:

In our previous investigation, the structures of the antibiotics, tuberactinomycins A and N were determined to be branched cyclopeptides in which γ-hydroxy-β-lysine was linked to the cyclic moieties as shown in Fig. 1.[2]

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



R = OH, Tuberactinomycin A
R = H, Tuberactinomycin N

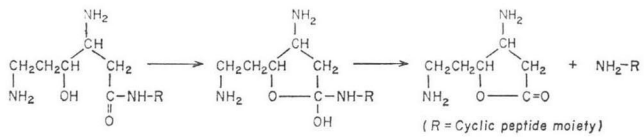
However, two different diastereoisomeric lactones of this amino acid, *i.e.*, *threo* and *erythro*-γ-hydroxy-L-β-lysine lactones were obtained, depending on condition for hydrolysis of the antibiotics. *Threo*-γ-hydroxy-β-lysine lactone was obtained after complete hydrolysis on heating with 6 M hydrochloric acid or after partial hydrolysis with concentrated hydrochloric acid at room temperature, while the *erythro* isomer was obtained after partial hydrolysis with concentrated sulfuric acid.[3] In either partial hydrolysis, only a single isomer was obtained being not contaminated with its diastereoisomer as far as it was liberated. The identity and configuration of these isomers were not only ascertained by physicochemical evidences, but also by comparison with synthetic samples.[4] We address ourselves here to the question of which configuration occurs in the intact antibiotics and what mechanism is involved in the inversion of the one isomer to the other.

For formation of two diastereoisomers there are two pathways, either a racemization at the β-carbon atom or an epimerization at the γ-carbon atom. In the sequential analysis of peptides, a retention of the configuration of β-carbon atom of β-hydroxy-α-amino acid in the *N*, *O*-acyl migration reaction has been accepted, and hitherto has escaped strict study.

According to the mechanism of *N*, *O*-acyl migration *via* cyclic carbinolamine as intermediate (Fig. 2), easy release of γ-hydroxy-β-lysine from tuberactinomycins A and N with concentrated acid is explicable. However, this mechanism can not lead to the formation of two diastereoisomers, one of which must be derived from the other isomer by inversion of one asymmetric center during the acid treatment.

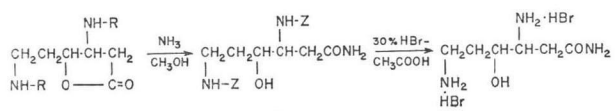
In order to clarify the rather anormalous results obtained and to determine the true configuration in the intact antibiotics, the behaviors of model derivatives of γ-hydroxy-β-lysine under acid conditions were investigated. Thus, the γ-hydroxy-β-lysine lactone **I** (*threo*

Fig. 2. Mechanism for liberation of γ-hydroxy-β-lysine lactone *via* cyclic carbinolamine intermediate with hydrochloric acid.



(R = Cyclic peptide moiety)

Fig. 3. Preparation of γ-hydroxy-β-lysine amide.

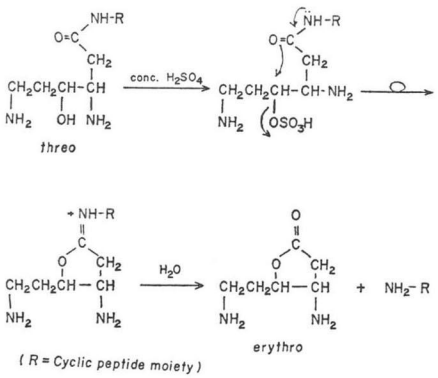


I R=H
II R=Z=$C_6H_5CH_2OCO$-
III
IV

and *erythro*) obtained from hydrolyzates was benzyloxycarbonylated and then converted to the amide derivatives **III** by ammonolysis. Debenzyloxycarbonylation of the product with 30% hydrogen bromide in glacial acetic acid afforded amide dihydrobromide **IV** (*threo* and *erythro*) with free β-hydroxy group respectively (Fig. 3). A simple hydroxy acid of **I** could not be obtained, since lactonization proceeded very rapidly for either isomer.

On hydrolysis of the amide dihydrobromides either by heating under reflux with 6 M hydrochloric acid or by treatment with concentrated hydrochloric acid at room temperature, *threo* **IV** gave the *threo* form of **I** and *erythro* **IV** yielded the *erythro* form of **I**. In other words, no configurational change occurred under these conditions. On the other hand, treatment of either amide **IV** with concentrated sulfuric acid followed by the complete hydrolysis with 6 M hydrochloric acid gave a mixture of *threo* and *erythro* **I**, though rich in the original form, indicating that configurational change must be ascribed to the procedure using concentrated sulfuric acid, although a liberation of the lactone from the amide occurred partially before the hydrolysis. This inversion never occurred with other mineral acids, *e.g.*, hydrochloric acid, hydrobromic acid, and anhydrous hydrogen fluoride. Hence, we now suggest a plausible mechanism involving S_{Ni} eliminationreact ion *via* *O*-sulfate and the cyclic intermediate for the acyl migration reaction in concentrated sulfuric acid.

Fig. 4. Mechanism for liberation of γ-hydroxy-β-lysine lactone with concentrated sulfuric acid accompanying an inversion of the configuration on γ-carbon atom.



This mechanism is consistent with our finding with threonine peptides as reported in previous papers.[5,6]

According to the above investigation, hydrolysis of the antibiotic with sulfuric acid liberates *erythro*-γ-hydroxy-β-lysine lactone with an inversion of the configuration on the γ-carbon atom of *threo*-γ-hydroxy-β-lysine residue as shown in Fig. 4.

Consequently, it was concluded that the true configuration of γ-hydroxy-β-lysine in tuberactinomycin A and N is L-*threo* which retained during hydrochloric acid hydrolysis and was inverted during concentrated sulfuric acid hydrolysis.

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