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AN INVERSION OF THREONINE TO ALLOTHREONINE IN THE N,O-MIGRATION REACTION OF THREONINE PEPTIDES IN CONCENTRATED SULFURIC ACID [1]

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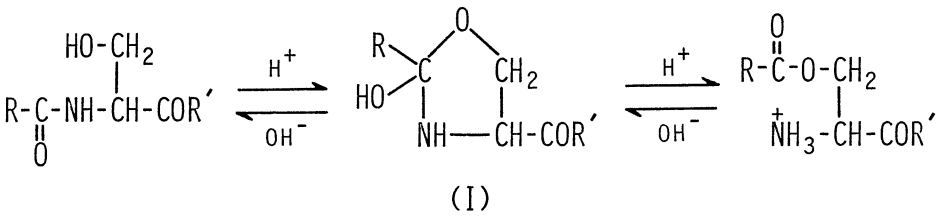
In a N,O-migration reaction of peptides containing β-hydroxy-α-amino acids in strong acids, it was generally accepted that the configuration on β-carbon atom is retained through the reaction. However, according to our recent investigation using concentrated sulfuric acid as strong acid, a configurational change was observed in the migration reaction. Thus, the threonine residue in peptides was converted to allothreonine residue on the reaction. For the migration reaction accompanying an inversion of configuration, we suggested the mechanism involving S_N2 elimination of O-sulfate of threonine residue.

For the selective chemical cleavage of peptide bonds containing hydroxy amino acids such as threonine or serine, the N,O-migration reaction in strong acids has been successfully applicable. Bergmann had first investigated on N,O-acyl migration which was revealed to proceed in reversible way depending on pH through a plausible intermediate, hydroxyoxazolidine(I) (Fig.1).[2] The



(I)

Fig.1. Mechanism of rearrangement *via* hydroxyoxazolidine(I).

mechanism *via* such cyclic intermediate has been accepted in many studies on the N,O-migration reaction in strong acids. For this reaction, concentrated

sulfuric acid has been most widely used.[3] Hydrogen chloride in methanol,[4] in ether,[5] or in nitromethane,[6] anhydrous phosphoric acid,[7] formic acid/boron trifluoride,[4] acetic anhydride in methanol,[8] and anhydrous hydrogen fluoride[9] are also useful for the same purpose.

In view of the stereochemistry, the configuration on β-carbon atom carrying hydroxyl group should be retained if the above mechanism is applied. In fact, Phillips and Baltzly[2b] have shown that ethyl DL-N-benzoylallothreoninate was converted into the corresponding O-benzoyl derivative without change of the configuration by action of ethanolic hydrogen chloride. Similar result was also obtained at the migration reaction of peptides in anhydrous hydrogen fluoride where no inversion occured on the β-carbon atom of the threonine residue.[9] On the contrary to these findings, there are known some migration reactions which involve the inversion of the configuration. This is the case, for example, in the conversion of N-acyl-(−)-ephedrine to O-acyl-(+)-Ψ-ephedrine by treatment in hydrochloric acid.[10] For the migration reaction by thionyl chloride, Elliott[3c] pointed out the possibility that an inversion of configuration may occur on an asymmetric carbon atom carrying the hydroxyl group. However, he could not decide which mechanism operates in the rearrangement of silk fibroin with concentrated sulfuric acid at that time.[3c]

From the results of our recent work, it now proved that a configuration on the β-carbon atom of threonine residue in peptides was always inverted on the migration reaction in concentrated sulfuric acid. Thus, the threonine residue in peptides listed in Table 1,[11] was converted to allothreonine residue in a fairly good yield in every case.

Table 1. Ratio of Inversion of Threonine Residue

Peptides*	Ratio (%)	
	Allothreonine	Threonine
Z-Ala-Thr-OH a)	78	22
H-Ala-Thr-OH a)	82	18
Z-Gly-Thr-OH a)	71	29
Z-Gly-Thr-OCH_3 a)	69	31
Z-Gly-Thr-Phe-OH b)	77	23
H-Gly-Thr-Phe-OH b)	77	23

* Used amino acids are of DL-forms in peptides a) and of L-forms in peptides b).

Paper chromatographic estimation of allothreonine and threonine was carried out as follows. Hydrolyzates of migration products were separated with paper chromatography using R-solvent (supernatant of *n*-butanol-water-acetone-28% ammonium hydroxide = 40 : 30 : 5 : 5) as a developing solvent. Ninhydrin was sprayed to the paper, and then the colored parts were cut off. These were extracted with water and then acetone, and the extract for each part was allowed to react with excess ninhydrin reagent. The solution was then diluted to a constant volume for colorimetry at 570 nm.

The N,O-migration reaction in concentrated sulfuric acid is presumably initiated by formation of O-sulfate. This mechanism is supported by the fact that O-sulfates of threonine and serine itself could be easily prepared and isolated as fine crystals in good yields under similar reaction condition to the migration reaction. Fasmann's observation[12] that the sulfate was formed before ester formation in the migration reaction of poly-DL-serine in concentrated sulfuric acid, is consistent with our assumption.

The next stage to the sulfate formation could be an elimination reaction of sulfate concomitantly followed by an inversion of the configuration on β-carbon atom carrying hydroxyl group according to a mechanism of S_N2 type as shown in Fig.2.[13]

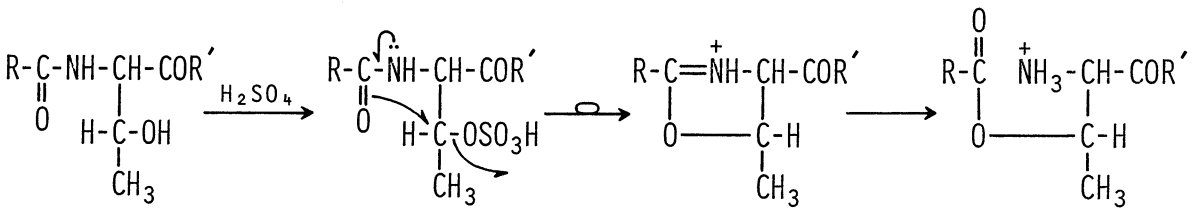


Fig.2. Proposed mechanism of migration reaction in concentrated sulfuric acid.

Incompleteness of the inversion of threonine residue in the peptides listed in Table 1 may mean that either simple hydrolysis preceded to the N,O-migration or the migration reaction did not proceed thoroughly. It has been reported that the acyl migration in concentrated sulfuric acid occurred only in a low yield particularly in threonine peptides,[9,14] and actually the slowness of the inversion reaction was recognized in our experiment too. Therefore, it could be concluded that the acyl migration in concentrated sulfuric acid is accompanied with complete inversion of the configuration on β-carbon atom of threonine residue resulting in the change to allothreonine residue once the

migration reaction takes place. From the results of our investigation, it was also confirmed that the inversion occurs only in concentrated sulfuric acid but not in another mineral acids, *i.e.*, hydrochloric acid, hydrobromic acid, and anhydrous hydrogen fluoride.

REFERENCES AND FOOTNOTES

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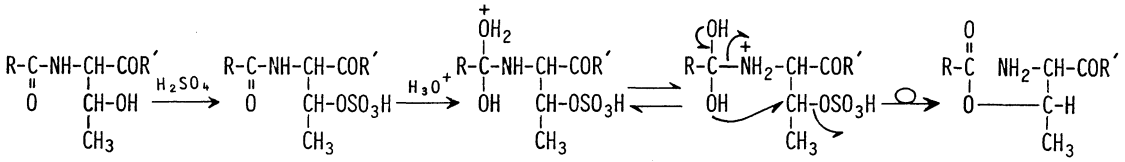
13. The following mechanism involving S_N2 substitution may also be considered as an alternative way.



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