99. Shoji Takemura : Chemical Studies on Antibiotics produced
by Actinomycetes. X. Racemomycin. (7).
Structure of Racemomycin-O.*¹

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It has been shown in previous papers of this series^{1,2)} that racemomycin-O gives β -lysine (I), roseonine (II), glucosamine (III), racemonic aldehyde (IV), carbon dioxide, and ammonia*³ as the degradation products by drastic hydrolysis with hydrochloric acid. The isolation procedure for these products is summarized in Chart 1.

Chart 1.

In order to clarify the linkage of these moieties in this antibiotic, following experiments were carried out.

Racemomycin-O was hydrolyzed under a mild condition with hydrochloric acid. The resulting mixture was developed by paper chromatography during the reaction. Only one spot of original antibiotic was observed at the beginning but later, many spots appeared gradually, and finally, only the spots of β -lysine, roseonine, glucosamine, and racemonic aldehyde were found (Fig. 1).

The undetermined, other Ninhydrin-positive substances were assumed as intermediates of hydrolysis and these were designated as intermediates-A, -B, and -C in the ascending order of their R_f values. Intermediate-C was extracted from paper strips

*¹ This is a part of a series entitled "Chemical Studies on Antibiotics produced by Actinomycetes" by H. Taniyama. Part IX. This Bulletin, 8, 574(1960).

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*³ The formation of ammonia was proved by Nessler's reagent.

1) S. Takemura : This Bulletin, 8, 154(1960).

2) *Idem* : *Ibid.*, 8, 574(1960).

1, Partial hydrolysates of racemomycin-O; 2, hydrolysates of intermediate-A; 3, hydrolysates of intermediate-B; 4, hydrolysates of intermediate-C; 5, hydrolysates of dinitrophenylated racemomycin-O; 6, hydrolysates of dinitrophenylated intermediate-A; 7, hydrolysates of dinitrophenylated intermediate-B; 8, hydrolysates of dinitrophenylated intermediate-C; 9, hydrolysates of HIO_4 -oxidation product of racemomycin-O; 10, hydrolysates of HIO_4 -oxidation product of intermediate-A; 11, hydrolysate of HIO_4 -oxidation product of intermediate-B; 12, hydrolysate of HIO_4 -oxidation product of intermediate-C.

Fig. 1. Paper Chromatogram of Partial Hydrolysates of Racemomycin-O and Hydrolysates of 2,4-Dinitrophenylated and Periodate-oxidized Products of Intermediates

with water and the extract was hydrolyzed again. The chromatogram (Fig. 1-4) showed that C is composed of roseonine and glucosamine.

The linking order of these two moieties in this intermediate could be decided in the following manner: The extract of spot C was concentrated, treated with 2,4-dinitro-1-fluorobenzene, and hydrolyzed again. The chromatogram of this hydrolysate is given in Fig. 1-8. N-(2,4-Dinitrophenyl)glucosamine and β -N-(2,4-dinitrophenyl)roseonine thereby formed were identified with synthesized samples in Rf values. The extract of intermediate-C has no reducing power.

On the basis of the above observations, the structure (VI) is proposed for the intermediate-C. Formation of such an intermediate is interesting in connection with formation of (V) as an intermediate of hydrolysis of roseothricin-A, one of the actinomycetes antibiotics studied by Goto, Hirata, *et al.*³⁾

Intermediate-B was extracted from paper strips, hydrolyzed, and afforded three Ninhydrin-positive spots of roseonine, β -lysine, glucosamine, and a small spot of intermediate-C. Then, by a similar manner, intermediate-B was dinitrophenylated and hydrolyzed to obtain unchanged glucosamine, β -N-(2,4-dinitrophenyl)roseonine, and β,ϵ -bis-

3) T. Goto, Y. Hirata, S. Hosoya, N. Komatsu: Bull. Chem. Soc. Japan, 30, 729(1957).

N-(2,4-dinitrophenyl)- β -lysine, which were identified with authentic samples (Fig. 1-7). From these results, the structure of intermediate-B must be as shown by (VII).

Intermediate-A afforded roseonine, β -lysine, glucosamine, and racemic aldehyde by rehydrolysis, while racemomycin-O itself also gave the same spots by complete hydrolysis (Fig. 1-2). Therefore, it could be concluded that substance A is a first product produced from the antibiotic, and ammonia and carbon dioxide were concurrently separated. This conclusion is supported by the fact that the evolution of carbon dioxide ceased when the original antibiotic disappeared on the paper chromatogram.

Similar test was applied to intermediate-A. The chromatogram (Fig. 1-6) of the hydrolysate after dinitrophenylation showed formation of β , ϵ -bis-N-(2,4-dinitrophenyl)- β -lysine, β -N-(2,4-dinitrophenyl)roseonine, free glucosamine, and racemic aldehyde.

This shows that racemomycin aldehyde is not linked to the amino groups which can be attacked by dinitrofluorobenzene. Therefore, the aldehyde must be linked to the hydroxyl group of roseonine or glucosamine moieties in acetal or semiacetal form.

Subsequently, the extract of intermediate-A was treated with periodate reagent and hydrolyzed. The roseonine spot disappeared but glucosamine was indicated on the chromatogram (Fig. 1-10). This shows that the presence of free hydroxyl group in roseonine moiety and the aldehyde is linked to hydroxyl group of either 3- or 4-position of glucosamine. It is probable that the structure of intermediate-A would be represented by (VIII) from above observations.

The remaining unknown part in the structure of racemomycin-O is the portion which forms ammonia and carbon dioxide by hydrolysis. From the analytical values of its salts, racemomycin-O is known to be a triacidic base and two out of three basic centers are in the β -lysine portion, as evidenced by the following series of experiments. Dinitrophenylation of racemomycin-O followed by hydrolysis results in formation of β , ϵ -bis-N-(2,4-dinitrophenyl)- β -lysine, free glucosamine, and racemic aldehyde, together with dinitrophenylated roseonine, formed from the dinitrophenylated intermediate-A. On the other hand, dinitrophenylated racemomycin-O gives free roseonine under the same conditions. The formation of free roseonine indicates that β -amino group of roseonine moiety is protected from the attack of the reagent and it is linked to $>\text{CO}$ group from which carbon dioxide is produced by hydrolysis. Racemomycin-O is inert to periodate and has no carboxylic group, according to potentiometric titration. Further, in the hydrolysis of racemomycin-O (Fig. 2), evolution of ammonia and carbon dioxide ceased at the time when the spot of racemomycin-O disappeared from the chromatogram and it may be assumed that these gases are produced at the same time during hydrolysis. It is thereby concluded that the $-\text{CO}-\text{NH}-$ linkage is bridged between amino and carboxyl groups in the roseonine part and the presumed structure of racemomycin-O would be formulated as (IX).

The yield of each of the degradation products of hydrolysis is extremely varied. This difference in yields is probably due to the interaction of unstable products which have amino or carbonyl group to produce black-colored polymers during the reaction. Accordingly, in order to elucidate this problem, variation of the products was followed semiquantitatively by paper chromatography.

The weighed racemomycin-O hydrochloride was refluxed with 6*N* hydrochloric acid in nitrogen stream and the mixture was chromatographed on paper during the reaction. Each spot was extracted from paper strips and colorimetrically determined. Fig. 2 shows the degree of stability in the descending order of β -lysine, roseonine, glucosamine, and racemomic aldehyde, and the order of their yield also agreed with this ranking.

Fig. 2. Pursuit Curve of Hydrolysis of Racemomycin-O
Ninhydrin-positive

O : Racemomycin-O A : Intermediate-A B : Intermediate-B
C : Intermediate-C R : Roseonine L : β -Lysine G : Glucosamine
Triphenyltetrazolium-positive Ra : Racemomic aldehyde

The structure of roseothricin-A has been formulated as (X) by Goto, Hirata, *et al.*³⁾ There is interest in comparing the structure of roseothricin-A and the newly presented structure (IX) for racemomycin-O.

Experimental

Partial Hydrolysis of Racemomycin-O—A mixture of 2 g. of racemomycin-O hydrochloride and 20 cc. of 3*N* HCl was heated under reflux for 5 hr. on a water bath. The paper chromatogram of the hydrolysate showed spots of β -lysine, roseonine, glucosamine, and racemonic aldehyde, and new spots of intermediates-A (Rf 0.03), -B (Rf 0.06), and -C (Rf 0.07) by the developing solvent of BuOH-AcOH-H₂O (4:1:5).

2,4-Dinitrophenylation of Intermediates—Five strips of paper developed from the above hydrolysate was extracted with water, concentrated *in vacuo* to 1 cc., and 0.2 cc. each of 5% solution of 2,4-dinitrofluorobenzene in EtOH was added. To this mixture 16 mg. of NaHCO₃ was added and each was mechanically shaken for 28 hr. The mixture was acidified with HCl, washed three times with Et₂O, and concentrated *in vacuo*. Each of these solutions, added with 1 cc. of 6*N* HCl, was heated in a sealed tube for 40 hr. at 100°, dried *in vacuo* over NaOH, and chromatographed on a filter paper. The chromatogram of these hydrolysates of 2,4-dinitrophenylated intermediates is given in Fig. 1-6, 7, and 8.

2,4-Dinitrophenylation of Racemomycin-O and Hydrolysis of the Product—About 10 mg. of racemomycin-O hydrochloride and 5 cc. of 50% EtOH were mixed, and 40 mg. of NaHCO₃ and 0.5 cc. of 5% EtOH solution of 2,4-dinitro-1-fluorobenzene were added. The mixture was shaken mechanically for 28 hr., acidified with HCl, washed three times with Et₂O, and dried *in vacuo* over NaOH. To this residue, 1.7 cc. of 6*N* HCl was added, the mixture was heated in a sealed tube for 40 hr. at 100°, and dried *in vacuo* over NaOH. The chromatogram is given in Fig. 1-5.

Hydrolysis of Periodate-oxidation Products of Intermediates and Racemomycin-O—i) Each extract obtained from 5 strips of paper developed as above from the hydrolysate of racemomycin-O was concentrated *in vacuo* to about 1 cc. and 1 drop of conc. HNO₃ and 1 cc. of 5% KIO₄ solution were added. After standing for 1 hr. at room temperature, 1 cc. of conc. HCl was added, the mixture was heated in a sealed tube for 40 hr. at 100°, and evaporated *in vacuo* to dryness. The chromatogram of these hydrolysates of periodate-oxidation intermediates-A, -B, and -C, is shown in Fig. 1-10, 11, and 12.

ii) Ten mg. of racemomycin-O hydrochloride was treated with HIO₄ by the method of Shriner, *et al.*⁴⁾ but no oxidation was observed. The mixture was evaporated *in vacuo* and heated with 1 cc. of conc. HCl in a sealed tube for 40 hr. at 100°. The chromatogram of this hydrolysate is shown in Fig. 1-9.

Hydrolysis of Racemomycin-O—Racemomycin-O hydrochloride (523 mg.) was dissolved in 6*N* HCl to make 50 cc. in total volume. The solution was refluxed in an oil bath and, at intervals of 0, 1, 4, 7, 10, 15, 20, 30, 40, and 45 hr. after heating, three 0.001-cc. portions of the hydrolysate were spotted on a filter paper and developed with BuOH-H₂O-AcOH (4:1:1). The chromatostrips were cut out at each spot after spraying Ninhydrin in BuOH-pyridine (5:1) or alkaline triphenyltetrazolium solution. Each paper strip was treated in a test tube with the above reagent in a water bath and the variations of the products was colorimetrically compared (Fig. 2).

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Summary

Racemomycin-O is composed of β -lysine, roseonine, glucosamine, racemonic aldehyde, carbon dioxide, and ammonia. The linkage of these moieties was examined mainly using paper chromatographic techniques. The proposed structure of racemomycin-O is formulated as (IX).

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4) P. L. Shriner, R. C. Fuson, D. Y. Curtin: "The Systematic Identification of Organic Compounds," 129(1956), John Wiley & Sons, Inc., New York.