The author is grateful to Dr. Satoru Kuwada, chief of the laboratories, for his encouragement throughout the present work and to Dr. Einosuke Omura for his valuable advices.

Summary

Utilizing the reaction of its secondary amino group, proline in proteins was determined in the presence of other amino acids. The material proteins were first hydrolyzed and then allowed to react with nitrous acid to convert the proline therein into N-nitrosoproline, when all the other amino acids were changed into the corresponding hydroxy acids. N-Nitrosoproline was then reduced to N-aminoproline with zinc dust and hydrochloric acid, and finally the N-amino compound was determined by azotometry by oxidation with potassium ferricyanide. Incidentally, the secondary amino group in the ring of tryptophan, histidine, and prolidine, and the guanidine group of arginine do not interfere with the present method.

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98. Shoji Takemura: Chemical Studies on Antibiotics produced by Actinomycetes. IX. Racemomycin. (6). Structure of New Degradation Product of Recemomycin-O.*1

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In the previous reports of this series,[1,2] it was shown that four degradation products, β-lysine (I), roseonine (II), glucosamine (III), and an unknown reducing substance were obtained from the hydrolysate of the new streptothricin-like antibiotic, racemomycin-O. Structural studies of the fourth compound were carried out and the results are presented in this paper.

On the paper chromatogram, this compound is found at Rf 0.05 (BuOH–AcOH–H_2O= 4:1:5), negative to Ninhydrin reagent and giving a red color by treatment with alkali solution of triphenyltetrazolium reagent. Therefore, this compound is a new degradation product which has never been discovered in hydrolysate of streptothricin-group antibiotics.

This substance often cannot be detected on paper chromatogram when the paper strips were exposed to the air before developing or spraying of reagent. This observation shows that this compound is sensitive to oxidation. Furthermore, this compound is negative to various amine reagents. In order to isolate this compound, the hydrolysate was continually extracted with ether and the extract was concentrated to obtain an oily residue. The residue was distilled under a reduced pressure and the oily substance which gave Rf 0.05 on paper chromatogram was obtained. The freshly distilled oil is positive to the Tollens, aniline hydrogenphthalate, ammoniac-silver nitrate, and fuchsin-

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*1 This constitutes Part IX of a series entitled "Chemical Studies on Antibiotics produced by Actinomycetes" by H. Taniyama.

*2 Kowakae, Fuse, Osaka-fu (竹村庄司).

1) H. Taniyama, S. Takemura: This Bulletin, 8, 150(1960).

2) S. Takemura: *Ibid.*, 8, 154(1960).

sulfur dioxide tests, and gives a phenylhydrazone, m.p. 178~181°. However, after standing for long hours, this substance changes into a substance negative to the above aldehyde tests. This changed oily material was soluble in alkaline water and showed obvious hydroxamic acid reaction. These observations show the presence of an aldehyde group in the fresh degradation product and it seems to be easily oxidized to the corresponding carboxylic acid. Accordingly, further structural studies were carried out on the oxidized carboxylic acid.

This reducing substance was designated as recemonic aldehyde and the corresponding carboxylic acid as racemonic acid.

Infrared absorption spectrum (Fig. 1) of racemonic acid shows typical bands of carboxylic acid. It was then derived to 4-bromophenacyl ester of white needles, m.p. 79~81°.

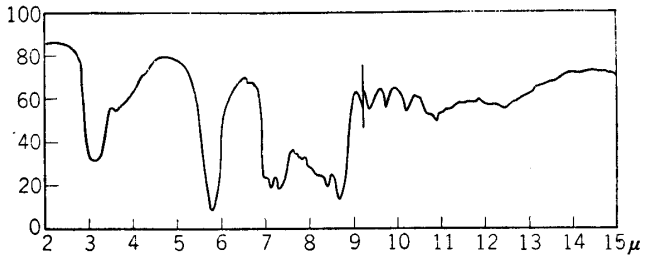


Fig. 1. Infrared Spectrum of Racemonic Acid (liq.)

Analytical data of the free acid and 4-bromophenacyl ester agreed well with the molecular formula of $C_6H_{12}O_4$.

Tests were made in order to clarify the linkage of remaining oxygen atoms in this acid and the substance was positive to the iodoform reaction, negative to Zeisel's alkoxyl test,[3] and negative to periodic acid oxidation.[4] Therefore, this acid will be represented by the partial formula (IV).

$$C_3H_4O \begin{cases} -COOH \\ -CH(OH)-CH_3 \end{cases} \quad (IV)$$

The remaining oxygen atom in the molecule should be linked as an ether and the relatively strong band observed in the infrared region at 1160 cm^{-1} (Fig. 1) is probably due to the ether C-O linkage. If the ether linkage is present in racemonic acid, the three structures, (V), (VI), and (VII), will be possible for this acid.

$$CH_3CH(OH)CH_2OCH_2CH_2COOH \quad (V)$$

$$CH_3CH(OH)CH_2CH_2OCH_2COOH \quad (VI)$$

$$CH_3CH(OH)CH_2OCH(CH_3)COOH \quad (VII)$$

To evidence the ether linkage, the acid was treated with a saturated solution of hydrogen bromide in acetic acid in a sealed tube. The reaction mixture was evaporated to dryness *in vacuo* and poured into conc. ammonia water. The paper chromatograms (Fig. 2) showed formation of β-alanine and 1,2-diaminopropane. The formation of these

3) P. L. Shriner, R. C. Fuson, D. Y. Curtin : "The Systematic Identification of Organic Compounds," 116(1956), John Wiley & Sons, Inc., New York.
4) *Ibid.*, 129.

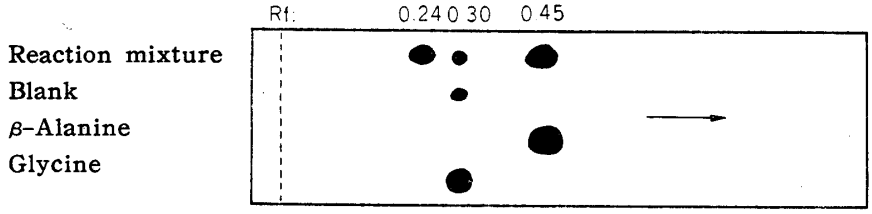
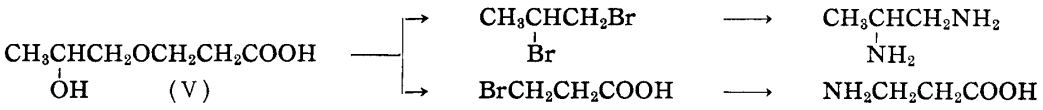
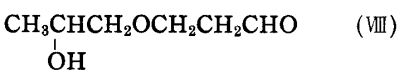


Fig. 2. Paper Chromatogram of Degradation Product of Racemonic Acid
(Ninhydrin, BuOH:AcOH:H_2O=4:1:5)

products indicates the presence of an ether linkage attached to the β-carbon atom of the carboxylic group in this acid. On the basis of these results, recemonic acid must be indicated by formula (V).



Further, the original aldehyde must have the structure (Ⅷ).



The synthesis of racemonic acid was then carried out by the route shown in Chart 1.

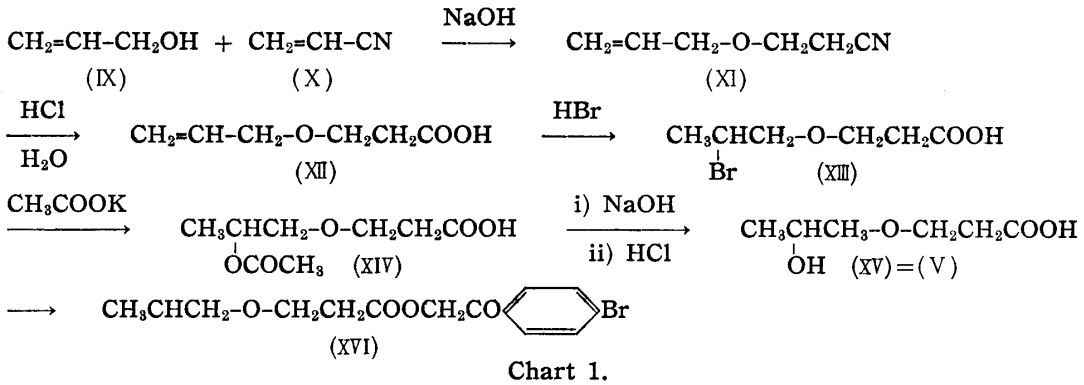


Chart 1.

Allyl alcohol was condensed with acrylonitrile by means of sodium hydroxide by the method of MacGregor and Pugh.[5] The nitrile (XI) was hydrolyzed with hydrochloric acid to obtain (XII) which was converted to (XIII) by addition of hydrogen bromide. The bromo acid (XIII) was heated with potassium acetate and the intermediate, (XIV), was hydrolyzed with sodium hydroxide, acidified, and extracted continually with ether. The residue of the ether extract was distilled at 118~120°/0.1 mm. Hg, and was converted to its 4-bromophenacyl ester, m.p. 80~81°, the mixed melting point of which with authentic sample showed no depression.

Racemonic acid obtained from the natural substance is optically inactive.

Experimental

Isolation of Racemonic Aldehyde (VIII)—A mixture of 20 g. of racemomycin-O hydrochloride and 50 cc. of 6*N* HCl was heated on a water bath for 32 hr. The reaction mixture was extracted continually with purified Et_2O for 24 hr., Et_2O layer was dried over Na_2SO_4, and evaporated. The residue was distilled under a reduced pressure; $b.p_{12}$ 110~114°(bath-temp.). Yield, ca. 30 mg. The syrup did not crystallize on chilling in a refrigerator overnight and, after standing for 10 days, it showed no reaction to aldehyde reagents.

5) J. H. MacGregor, C. Pugh : J. Chem. Soc., **1945**, 535.

Racemonic Acid (V)—The syrupy substance which had been allowed to stand in an open flask was treated with one drop of 3% H_2O_2 and, after 5 hr., the mixture was dried and distilled under a reduced pressure; b.$p_{0.1}$ 118~121°; $[\alpha]_D^{17}$ 0.1° (c=1.0, EtOH), 0.0° (c=3, EtOH). Yield, ca. 30 mg. (syrup). This substance is positive to iodoform and hydroxamic acid reactions, and negative to Ninhydrin, triphenyltetrazolium, Tollens, and Fehling tests. It is soluble in Me_2CO, Et_2O, $CHCl_3$, benzene, and alkaline water. *Anal.* Calcd. for $C_6H_{12}O_4$: C, 48.65; H, 8.11. Found: C, 48.72; H, 8.52.

Phenylhydrazone of Racemonic Aldehyde—About 10 mg. of (VIII) was dissolved in 0.5 cc. of 50% MeOH and 5 drops of a mixture of 0.1 cc. of phenylhydrazine, 0.5 cc. of AcOH, and 0.5 cc. of water was added. The separated phenylhydrazone was collected, washed with a small volume of ice water, and dried to white needles, m.p. 178~181°. *Anal.* Calcd. for $C_{12}H_{18}O_2N_2$: C, 68.86; H, 8.11; N, 12.61. Found: C, 69.01; H, 8.23; N, 12.77.

4-Bromophenacyl Ester of Racemonic Acid—About 20 mg. of the acid (V) and 0.5 cc. of water were brought to pH 7.0 by addition of *N* NaOH, dried *in vacuo*, and the residue was dissolved in 1 cc. of EtOH. To this solution, 50 mg. of 4-bromophenacyl bromide was added, the mixture was heated under reflux for 2 hr., allowed to stand overnight, and filtered. The filtrate was concentrated to 1/2 the original volume and separated colorless needles, m.p. 79~81°, were recrystallized from EtOH. *Anal.* Calcd. for $C_{14}H_{17}O_5Br$: C, 48.70; H, 4.92. Found: C, 48.68; H, 4.99.

Degradation of Racemonic Acid—A mixture of 10 mg. of the acid (V) and 0.5 cc. of AcOH saturated with HBr, sealed in a glass tube, was heated for 48 hr. in an oil bath. After cool, the mixture was pipetted out, evaporated to dryness *in vacuo*, and dissolved in 2 drops of water. The paper chromatograms of the products are shown in Fig. 2.

Synthesis of Racemonic Acid—i) 3-(Allyloxy)propionitrile (XI): A mixture of acrylonitrile (X) and 20 g. of allyl alcohol (IX), added with 21 cc. of 2% NaOH, was shaken until a slight evolution of heat ceased. The upper layer was separated, neutralized with AcOH, dried over Na_2SO_4, and distilled. The nitrile was obtained as a clear colorless liquid, b.p. 192°; yield, 37 g. *Anal.* Calcd. for C_6H_9ON: C, 64.86; H, 8.11; N, 12.61. Found: C, 64.91; H, 8.50; N, 12.38.

ii) 3-(Allyloxy)propionic Acid (XII): A mixture of 3-(allyloxy)propionitrile (XI) (9 g.) and 30 cc. of 35% HCl was boiled for 1 hr. After cool, 10% NaOH was added and the alkaline solution was extracted with two 30-cc. portions of Et_2O. The aqueous layer was acidified with 10% HCl and extracted continually with Et_2O. The Et_2O layer was separated, dried over Na_2SO_4, filtered, and the residue obtained on evaporation of Et_2O was distilled under a reduced pressure, collecting the fraction of b.p. 192°; yield, 4 g. *Anal.* Calcd. for $C_6H_{10}O_3$: C, 60.01; H, 13.89. Found: C, 60.23; H, 14.12.

iii) 3-(2-Bromopropoxy)propionic Acid (XIII): HBr was bubbled through 2 g. of 3-(allyloxy)propionic acid (XII) under strong cooling and the mixture was allowed to stand in a refrigerator overnight. Excess HBr was evaporated on a water bath *in vacuo*, 5 cc. of water was added, and distilled again under a reduced pressure. The syrupy residue was extracted with two 10-cc. portions of Et_2O, the extract was distilled, and a fraction (1.9 g.) of b.p_{15} 152~153° was collected. *Anal.* Calcd. for $C_6H_{11}O_3Br$: C, 34.14; H, 5.22; Br, 37.89. Found: C, 34.02; H, 5.51; Br, 37.76.

iv) Racemonic Acid (3-(2-Hydroxypropoxy)propionic Acid) (XV=V): A mixture of 1.5 g of (XIII) and 2.0 g. of anhyd. AcOK was heated in an oil bath for 4 hr. at 100°. The reaction mixture was poured into 20 cc. of 10% NaOH, the mixture was heated on a water bath for 1 hr., acidified with 10% HCl, and extracted continually with Et_2O for 24 hr. The Et_2O extract was dried over Na_2SO_4 and fractionated *in vacuo*; b.$p_{0.1}$ 118~120°; yield, 0.5 g. This oil was identical in infrared spectrum with that of the natural product. The acid was converted to 4-bromophenacyl ester of m.p. 79~81° and admixture of this ester with that of an authentic sample showed no depression.

The author is deeply indebted to the members of the Research Laboratory of Dainippon Pharmaceutical Co. for elemental analysis and determination of infrared spectra, and to Mr. N. Fukida, University of Osaka, for a part of the analysis. Thanks are also expressed to Miss Ai Nakanishi for her technical help.

Summary

Racemonic acid, obtained from the hydrolysate of racemomycin-O in company with β-lysine, roseonine, and glucosamine, was studied. First, the corresponding aldehyde, racemonic aldehyde, was isolated from the ether extract and the acid was obtained as an oxidation product of the aldehyde. Structural studies of this acid were carried out and its structure was determined as (V) by degradation and synthesis. On the basis of these results, racemonic aldehyde (VIII) was found to be a new component of this antibiotic.

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