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30. **Shoji Takemura**: Racemomycin (5). Degradation Products of Racemomycin-O.*[1]

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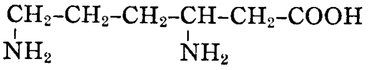
The fermentation of *Streptomyces racemochromogenus* (Shinobu-strain) and the isolation of new antibiotic, racemomycin-O, from its fermented broth were described in the preceding paper of this series.[1]

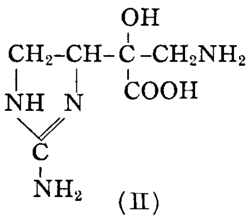
In this report the investigations on some of acid hydrolysates of the antibiotic are described. As reported previously on the paper chromatogram of the hydrolysate, three ninhydrin-positive substances and two spots which were positive for triphenyltetrazolium reagent were detectable, and one of the ninhydrin-spots was identical with one of the tetrazolium-spots in Rf values. In conclusion, four paper chromatographically detectable substances, two of basic, one basic-reducing and one reducing substances will be isolated from the hydrolysate. From Rf values of two ninhydrin-positive substances, it is supposed that these are β-lysine (I) and roseonine (II) which have been found from the hydrolysate of racemomycin-B[2] and from other streptothricin-group antibiotics, e.g. roseothricin,[3] streptolin,[4] streptothricin,[5] and geomycin,[6] as hodrolysates.

In order to clarify this supposition, attempt was made to isolate these substances. The hydrolysate was concentrated *in vacuo* to a syrup, dissolved in water, and precipitated as a picrate. The mixture of solid picrates was fractionally recrystallized from hot water and two kinds of picrates, m.p. 204° and m.p. 242°(decomp.), were separated. The former was identical with the picrate of β-lysine, and the latter was identical with that of roseonine from the mixed melting points, infrared spectra, and Rf values.



$\underset{NH_2}{CH_2}\text{-}CH_2\text{-}CH_2\text{-}\underset{NH_2}{CH}\text{-}CH_2\text{-}COOH$

(I)



(II)

Experiments were conducted concerning ninhydrin-positive reducing substance of Rf 0.12. Considering the other streptothricin-group antibiotics, streptolin-B and streptothricin,[7] it has been reported that D-gulosamine (2-deoxy-2-amino-D-gulose) (III) was isolated from these hydrolysates.

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*[1] This constitutes Part VIII of a series entitled "Chemical Studies of 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] *Idem.*: Yakugaku Zasshi, **77**, 1215(1957).

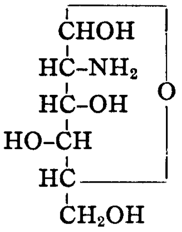
[3] K. Nakanishi: J. Antibiotics (Japan), **7**, Ser. B., 229(1954); K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto, Y. Hirata: Bull. Chem. Soc. Japan, **27**, 539(1954); K. Nakanishi, T. Ito, Y. Hirata: J. Am. Chem. Soc., **76**, 2845(1954).

[4] E. Van Tamelen, E.E. Swissman: J. Am. Chem. Soc., **74**, 3713(1952); *Ibid.*, **75**, 2031(1953).

[5] H.E. Carter, W.R. Herm, E.M. Lausford, Jr., A.C. Dage, Jr., N.P. Salzman, D. Shapiro, W. R. Taylor: *Ibid.*, **74**, 3704(1952); H.E. Carter, R.K. Clark, Jr., P. Kohn, J.W. Rothrock, W.R. Taylor, C.A. West, G.B. Whilfield, W.G. Jackson: *Ibid.*, **76**, 566(1954).

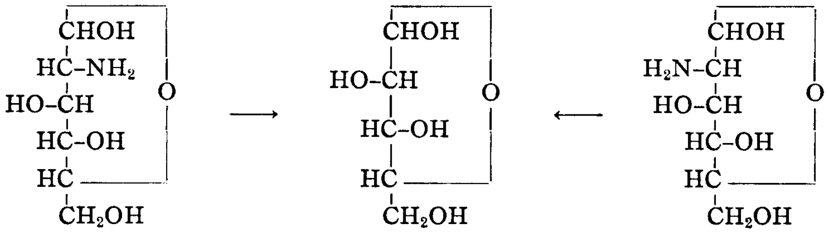
[6] H. Brockmann, H. Musso: Chem. Ber., **88**, 648(1955).

[7] E. Van Tamelen, J.R. Dyer, H.E. Carter, J.V. Pierce, E.E. Daniels: J. Am. Chem. Soc., **78**, 4817(1956).


(III)

Moreover, it was reported by Goto, *et al.*[8] that roseothricin-A also contains a hexosamine as its component, although no direct comparison between the two has yet been made, it may be safe to regard this hexosamine as identical with gulosamine (III).

2-Aminohexoses, e.g. chondrosamine, glucosamine, and gulosamine showed similar Rf values on paper in commonly used developing solvents; for instance 0.25; 0.33; 0.33 (BuOH:AcOH:H_2O, 4:1:1), 0.35; 0.43; 0.44(collidine), 0.64; 0.70; 0.74(PhOH:NH_3)[9] respectively. Therefore, many methods have been proposed for determining hexosamines by paper chromatography. Gardell, *et al.*, Stoffyn, *et al.*, and Villegas, *et al.*[7,10] showed that hexosamine is oxidized with ninhydrin to corresponding pentoses, e.g., lyxose from chondrosamine, xylose from gulosamine, and arabinose from glucosamine. From the part of Rf 0.12 of the developed paper strip (BuOH:AcOH:H_2O, 4:1:5) unknown amino sugar was extracted with water and heated with ninhydrin in dilute pyridine. The purple precipitate was filtered off and the filtrate was concentrated *in vacuo* to a small volume. This concentrate showed Rf 0.21 (BuOH:AcOH:H_2O, 4:1:5), which was compared with other pentoses and was found to be identical with that of arabinose (VI). These evidences show that the original amino sugar must be either glucosamine (IV) or mannosamine (V).


(IV) (VI) (V)

On the other hand, Chargaff and Bovarinick showed that D-gulcosamine gives extremely insoluble N-benzoyloxycarbonyl derivative by treating with benzoyloxycarbonyl chloride.[11] Therefore, the hydrolysate of racemomycin-O which had been freed from β-lysine and roseonine as picrates, was treated with benzoyloxycarbonyl chloride and the precipitated material was recrystallized to a pure state. The mixed melting point with authentic sample showed no depression and $[\alpha]_D$ values of both samples were also identical.

The remaining moiety of racemomycin-O which was detected by paper chromatography as triphenyltetrazolium-positive spot was also isolated from the hydrolysate mixture by ether extraction. The structural studies on this product will be reported in the following paper.

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8) T. Goto, Y. Hirata, S. Hosoya, N. Komatsu : Bull. Chem. Soc. Japan, **30**, 729(1957).
9) *Idem.* : *Ibid.*, **30**, 304(1957).
10) S. Gardell, *et al.* : Acta Chem. Scand., **4**, 970(1950); P. J. Stoffyn, *et al.* : Arch. Biochem. Biophys., **52**, 373(1954); R. Villegas, G. Villegas : Acta Cient. Venezolana : **8**, 19(1957).
11) E. Chargaff, M. Bovarinick : J. Biol. Chem., **118**, 421(1937).

Experimental

Hydrolysis of Racemomycin-O—Racemomycin-O hydrochloride (15 g.) was heated with 6*N* HCl (120 cc.) in an oil-bath for 48 hr. The condenser was fitted with a trap which contained $Ba(OH)_2$ solution to catch CO_2 evolved. After about 3 hr., evolution of CO_2 ceased. $BaCO_3$ was filtered and weighed (3.9 g.). The hydrolysate was filtered, concentrated *in vacuo* to a syrup, dissolved in 100 cc. of water, evaporated *in vacuo*, and dried over $CaCl_2$.

Isolation of β-Lysine and Roseonine—The mixture of hydrolysate was dissolved in 50 cc. of water and 50 cc. of hot saturated solution of picric acid was added. After standing overnight, the mixture of picrate was collected and fractionated by recrystallization from water to two kinds of picrate; m.p. 204°(2 g.) and m.p. 242°(decomp.)(1.7 g.). These picrates were respectively identical with the picrates of β-lysine (m.p. 204°) and roseonine (m.p. 242°) in mixed melting points and infrared spectra.

Identification of Amino Sugar with Glucosamine—1) Paper chromatography : The paper strips of the hydrolysate which were developed by $BuOH:AcOH:H_2O(4:1:5)$, was cut off at Rf 0.12, extracted with water, and the extract was evaporated *in vacuo* to dryness. A part of the residue was dissolved in 0.2 cc. of water and 0.1 cc. of pyridine, 10 mg. of ninhydrin was added and warmed on a water bath. After 30 min., the reaction mixture was filtered and the filtrate was decolorized with charcoal. The filtrate was evaporated to dryness *in vacuo*, and the residue was chromatographed on paper.

2) Benzoyloxycarbonyl Derivative : The filtrate, freed from β-lysine and roseonine picrate, was treated with 5 cc. of conc. HCl, extrated with three 20-cc. portions of ether. The aqueous layer was concentrated *in vacuo* to a syrup, dissolved in 10 cc. of water, and evaporated again. The residue was mixed with 10 cc. of water, and 2.0 g. of $NaHCO_3$ and 2.0 g. of benzoyloxycarbonyl chloride (in two portions) were added, and the mixture was shaken for 45 min. After chilling in a refrigerator, the precipitate was collected and thoroughly washed with ice water. On recrystallization from MeOH, white needles were obtained which melted with decomposition at 213°; yield, 0.6 g., $[\alpha]_D$ 75.0°(in pyridine). The mixed m.p. with the authentic sample showed no depression.

The author is indebted to Prof. Dr. S. Aoyama for providing him with facilities for this study.

Summary

As the degradation products of recemomycin-O, β-lysine (I), roseonine (II), and D-glucosamine (IV) were obtained. β-Lysine and roseonine were isolated as picrates and the amino sugar was identified by paper chromatography and isolated as N-benzoyloxycarbonyl derivative which was identical with the authentic N-benzoyloxycarbonyl-D-glucosamine in mixed melting point and optical rotations.

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