The better conditions for the synthesis of labeled CM and the kinetic studies will be reported in the near future.

Addendum. In the course of this study the authors acknowledged unexpectedly the paper[6] in which the exchange reaction of labeled CM in aqueous medium was reported.

National Institute of Hygienic Sciences,
Kamiyoga, Setagaya-ku, Tokyo

AKIRA TANAKA
GORO URAKUBO

Received June 5, 1968

6) E. Hallaba and H. El-Asrag, *J. Nuclear. Med.*, **8**, 686 (1967).

[Chem. Pharm. Bull. 16(10)2086—2088(1988)]

UDC 615.332.011.5 : 576.852

Total Synthesis of *dl*-Anisomycin

The antibiotic anisomycin,[1] a fermentation product of various species of *Streptomyces*, has been shown to have widespread activity against pathogenetic protozoa and fungi, notably *Trichomonas vaginalis, Endamoeba histolytica* and *Candida albicans*,[2] and has already been used for the treatment of amebic dysentary.[3] Chemical studies[4] on anisomycin and successive X-ray investigations[5] have elucidated its structure as 2α-*p*-anisylmethyl-3α-acetoxy-4β-hydroxypyrrolidine as shown in **1**.[6] Recently, Wong reported that the absolute configuration of anisomycin should be 2R, 3S, 4S by its chemical correlation with a transformation product obtained from natural L-tyrosine.[7] The present paper describes a total synthesis of *dl*-anisomycin from DL-tyrosine.

Following the procedure of McKinney, *et al.*,[8] cyanoethylation of DL-tyrosine gave N-(2-cyanoethyl)-DL-tyrosine,[9] mp $> 270°$, which was further treated with hydrochloric acid in ethanol to yield N-(2-carbethoxyethyl)-DL-tyrosine ethyl ester (**2a**), mp 71—72.5°. N-Acetylation of **2a** with acetic anhydride in methanol, followed by O-methylation of the resulting N-acetate of mp 126—128° with dimethyl sulfate, afforded N-(2-carbethoxyethyl)-N-acetyl-O-methyl-DL-tyrosine ethyl ester (**2b**) as a viscous oil. Dieckmann cyclization of **2b** with sodium hydride in benzene and successive decarboxylation with hydrochloric acid in aqueous acetic acid gave a syrupy 1-acetyl-2-*p*-anisylmethylpyrrolidin-3-one (**3a**), which formed a tosylhydrazone (**3b**) of mp 218° (decomp.). Alkaline decomposition of the

1) B.A. Sobin and F.W. Tanner, Jr., *J. Am. Chem. Soc.*, **76**, 4053 (1954).
2) J.E. Lynch, A.R. English, H. Bauck, and H. Deligianis, *Antibiot. Chemotherapy*, **4**, 844 (1954).
3) A.B. Cue and J.G.H. Diaz, *Rev. Invest. Bol. Univ. Guadalajara*, **1**, 94 (1961); G.G. Miranda and E. Urbina, *ibid.*, **1**, 95 (1961); J.A. Portilla, *ibid.*, 1, 95 (1961); A.A. Plata, H.B. Zapata, and V.A. Munoz, *ibid.*, **1**, 96 (1961).
4) J.J. Beereboom, K. Butler, F.C. Pennington, and I.A. Solomons, *J. Org. Chem.*, **30**, 2334 (1965).
5) J.P. Schaefer and P.J. Wheatley, *J. Org. Chem.*, **33**, 166 (1968).
6) Recently, Grollman has reported that anisomycin blocks the aminoacyl-sRNA transfer reaction to the polyribosome in protein biosynthesis in a manner similar to ipecac alkaloids and has proposed an interesting prediction on their structural basis for the inhibition of protein synthesis. See A.P. Grollman, Abstracts 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967, M2.
7) C.M. Wong, *Can. J. Chem.*, **46**, 1101 (1968).
8) L.L. McKinney, W.H. Uhing, E.A. Setzkorn, and J.C. Cowan, *J. Am. Chem. Soc.*, **72**, 2599 (1950); *Idem, ibid.*, **74**, 5183 (1952).
9) All compounds were characterized by infrared, ultraviolet and nuclear magnetic resonance spectra and elementary analysis.

tosylhydrazone (**3b**) in diethylene glycol yielded 2-*p*-anisylmethyl-Δ^3-pyrroline (**4a**) of bp 115—116° (0.4 mmHg). The yield of the transformation of DL-tyrosine into **4a** was 23%.

With work up as described in our previous communication,[10] epoxidation of a N-carbobenzyloxy derivative (**4b**) of **4a** was carried out by treatment with pertrifluoroacetic acid in the presence of sodium carbonate in boiling dichloromethane, furnishing an α-epoxide[11] (**5**) and a β-epoxide (**6**) in a total yield of 40%. Each component was isolated by silica gel column chromatography. The structure of these epoxides (**5** and **6**) were confirmed by the following facts: the major β-epoxide (**6**) was treated with acetic acid in the presence of sodium acetate to yield, with selective epoxide ring opening, one syrupy isomer (**7a**) of monoacetates, along with a syrupy diacetate (**7b**). Mesylation of **7a** in pyridine, followed by treatment of the resulting acetoxymesylate (**7c**) with ethanolic potassium hydroxide solution, afforded the minor epimeric epoxide (**5**) in a good yield.

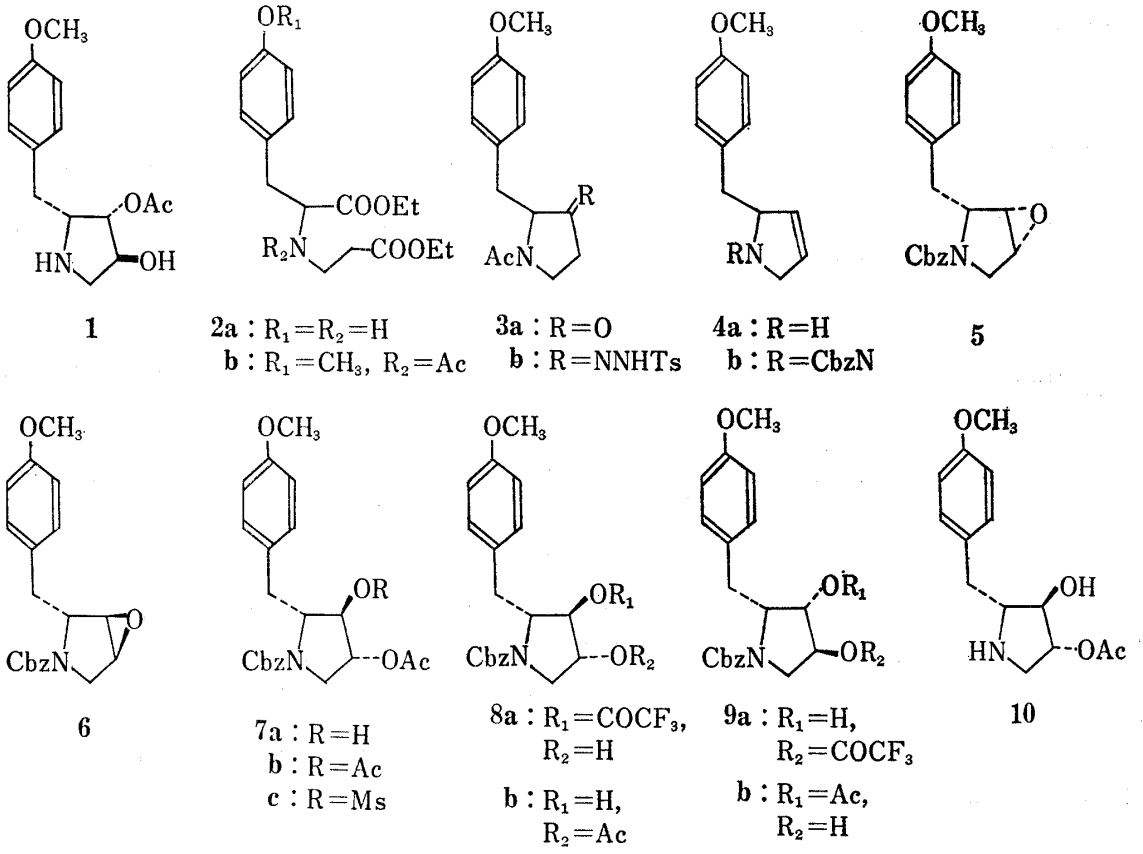

Chart 1

On the other hand, analogous treatment of **5** with trifluoroacetic acid gave an unseparable mixture of 1-carbobenzyloxy-2α-*p*-anisylmethyl-3β-trifluoroacetoxy-4α-hydroxy-(**8a**) and-3α-hydroxy-4β-trifluoroacetoxypyrrolidine (**9a**). Acetylation of the mixture of **8a** and **9a** with acetic anhydride in pyridine and successive removal of trifluoroacetyl group by treatment with water afforded a mixture of 3β-hydroxy-4α-acetoxypyrrolidine (**8b**) and 3α-acetoxy-4β-hydroxypyrrolidine (**9b**), which also could not be separated into the respective components. Removal of the carbobenzyloxy group from the mixture of **8b** and **9b** by hydrogenation over palladium charcoal afforded 2α-*p*-anisylmethyl-3β-hydroxy-4α-acetoxypyrrolidine

10) S. Oida and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1637 (1968).

11) Relative stereochemical relationships of the substituents on pyrrolidine ring of all compounds were deduced from the known structure of natural anisomycin (1), based on their mechanistic rationalizations, and also partly supported by analysis of their nuclear magnetic resonance spectra.

(**10**) of mp 147—148.5° (17% yield from **5**) and 2α-*p*-anisylmethyl-3α-acetoxy-4β-hydroxypyrrolidine (**1**) of mp 119.5—121°[12] (11% yield from **5**), which were separated by silica gel column chromatography. **10** was identified with the sample obtained by removal of the carbobenzyloxy group from **7a**. **1** exhibited the same infrared and nuclear magnetic resonance spectra and the same behavior on thin-layer chromatogram as the sample of natural anisomycin.[13] The synthetic *dl*-anisomycin showed half the activity against *Candida albicans*, as compared to that of the natural antibiotic.

Acknowledgement We are indebted to Dr. G. Sunagawa, Director, Drs. I. Iwai and H. Okazaki, Assistant Directors of our laboratories, for their encouragement, to Dr. M. Shirasaka and Mr. K. Furuya for the microbiological assay and to Mr . Y. Ohashi for his technical assistance.

Central Research Laboratories,
Sankyo Co., Ltd.,
Hiromachi, Shinagawa-ku, Tokyo

SADAO OIDA
EIJI OHKI

Received June 17, 1968

12) Repeated recrystallization of **1** from benzene–hexane gave polymorphic crystals which showed a higher, but not-sharp melting point.
13) The sample of anisomycin was supplied by Dr. L. Delcambe, International Center of Information on Antibiotics, Liége, Belgium, to whom we wish to express our appreciation.

[Chem. Pharm. Bull. 16(10)2088—2089(1968)]

UDC 615.398.12 : 612124

On the Third Active Peptide on Smooth Muscle in the Skin of *Rana nigromaculata*

HALLOWELL

In the previous communication, occurrence of two kinds of peptide, val[1]–thr[6]–bradykinin and bradykinin, has been demonstrated in the skin of *Rana nigromaculata*.[1]

The present communication describes the purification and amino acid sequence of the third peptide (peptide III). Separation of the peptide III from the bradykinin fraction, which corresponded to active fraction II in the previous communication,[1] was carried out as shown in chart I.

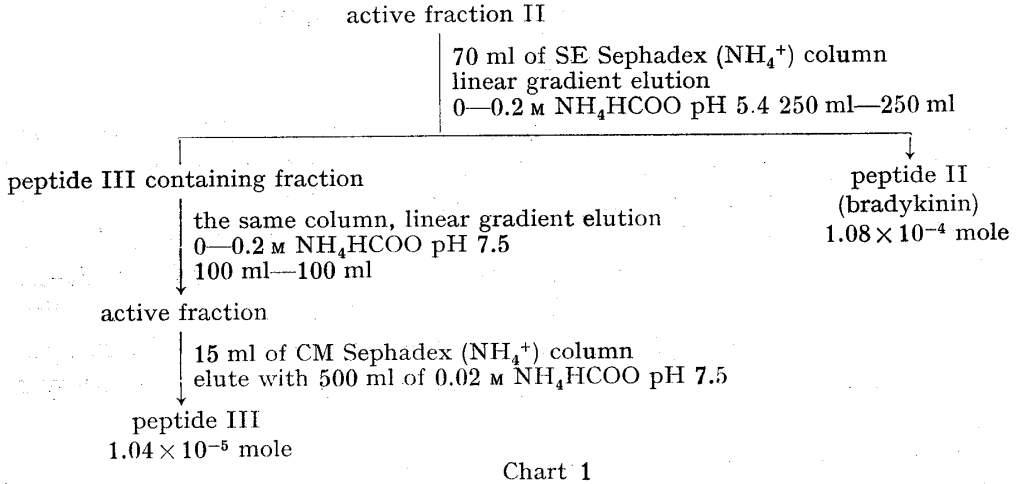

Chart 1

1) T. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **16**, 769 (1968).