[Chem. Pharm. Bull. 13(5) 599~602 (1965)]

UDC 547.466.2.02 : 615.779.9

81. Shoshiro Nakamura, Takeo Chikaike, Hiroshi Yonehara, and Hamao Umezawa : Isolation, Characterization and Structural Elucidation of New Amino Acids from Bottromycin A.

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Bottromycin A ($C_{41}H_{62}O_7N_8S$) and B ($C_{40}H_{60}O_7N_8S$) are antibiotics obtained from a cultured-broth of Streptomyces No. 3668-L2 and their physico-chemical and biological properties were reported in the former paper.[1] Hydrochloric acid hydrolysis of bottromycin A[1] yields each one mole of 3-methyl-3-phenylalanine, 2-amino-3,3-dimethylbutyric acid, valine, 3-(2-thiazolyl)-β-alanine, *cis*-3-methylproline and glycine, and the hydrolysis of bottromycin B yields the same amino acids except proline instead of *cis*-3-methylproline.[1]

3-Methyl-3-phenylalanine and 3-(2-thiazolyl)-β-alanine were isolated from bottromycin by Waisvisz, *et al.*[2~5] in 1957. 2-Amino-3,3-dimethylbutyric acid and *cis*-3-methylproline are new amino acids found by the present authors in natural products.

This paper reports on isolation, characterization and structural studies of the amino acids, particularly the new amino acids, in bottromycin A.

The structural studies of bottromycin A and B will be presented in the next paper.[6]

Bottromycin A was decomposed with acetic anhydride[1,5] at 100° for 3 hour, and after N-(N-acetyl-3-methyl-3-phenylalanyl)-3-(2-thiazolyl)-β-alanine methyl ester was removed by filtration. The filtrate was evaporated and the residue hydrolyzed with constant boiling hydrochloric acid for 24 hour.

Rf values of the amino acids in the hydrolyzate by paper chromatography are shown in the Table I.

Table I. Rf Values of the Amino Acids in Bottromycin A

	3-MePheAla.	DMAB	Val.	3-MePro.	Thia-β-Ala.	Gly.
Solv. I	0.64	0.57	0.50	0.46	0.38	0.23
Solv. II	0.68	0.60	0.52	0.46	0.40	0.20
Solv. III	0.62	0.55	0.43	0.38	0.36	0.15

DMAB : 2-Amino-3,3-dimethylbutyric acid
Solv. I : PrOH-H_2O=7:3
Solv. II : iso-BuOH-HCOOH-H_2O=70:15:13
Solv. III : BuOH-AcOH-H_2O=100:12:100 (upper phase)

The hydrolyzate was subjected to a partition chromatography on a cellulose powder column with butanol-acetic acid-water=100:12:100 (upper phase). The amino acids were eluated from the column in the order as follows : 3-methyl-3-phenylalanine, 2-amino-3,3-dimethylbutyric acid, valine, *cis*-3-methylproline, 3-(2-thiazolyl)-β-alanine and glycine.

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1) S. Nakamura, T. Chikaike, K. Karasawa, N. Tanaka, H. Yonehara, H. Umezawa : J. Antibiotics, **9**, 47 (1965).
2) J.M. Waisvisz, M.G. van der Hoeven, J. van Peppen, W.C.M. Zwennis : J. Am. Chem. Soc., **79**, 4520 (1957).
3) M.J. Waisvisz, M.G. van der Hoeven, J.F. Holsher, B. te Nijenhuis : *Ibid.*, **79**, 4522 (1957).
4) J.M. Waisvisz, M.G. van der Hoeven, B. te Nijenhuis : *Ibid.*, **79**, 4524 (1957).
5) J.M. Waisvisz, M.G. van der Hoeven : *Ibid.*, **80**, 383 (1957).
6) S. Nakamura, T. Chikaike, H. Yonehara, H. Umezawa : J. Antibiotics, **9**, 60 (1965).

An amino acid, $C_{10}H_{13}O_2N$ white needles m.p. 186~188°, $[\alpha]_D^{18}$ −31 (c=1, H_2O), was identified as 3-methyl-3-phenylalanine by a paper chromatography with an authentic specimen obtained from bottromycin and by its nuclear magnetic resonance spectrum. The infrared absorption spectrum is shown in Fig. 1.

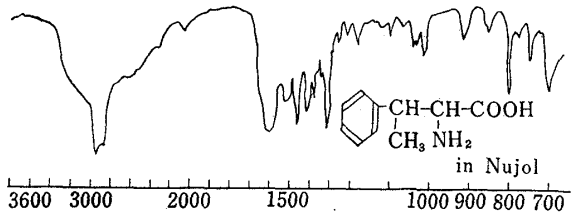

Fig. 1. Infrared Spectrum of 3-Methyl-3-phenylalanine (in Nujol)

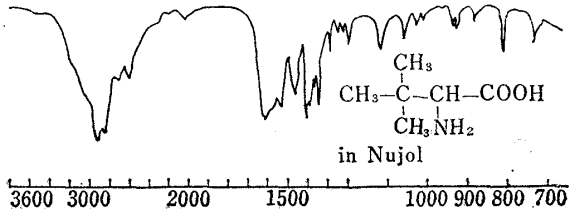

Fig. 2. Infrared Spectrum of 2-Amino-3,3-dimethylbutyric Acid (in Nujol)

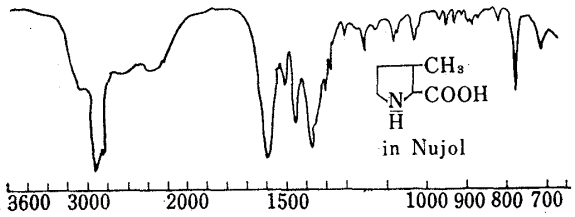

Fig. 3. Infrared Spectrum of *cis*-3-Methylproline, m.p. 218~222° (in Nujol)

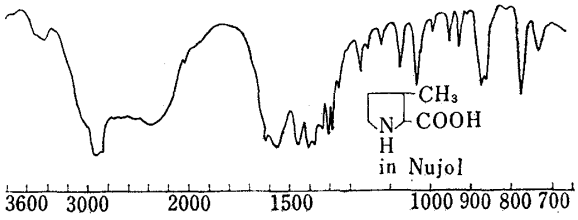

Fig. 4. Infrared Spectrum of *cis*-3-Methylproline, m.p. 241~244° (in Nujol)

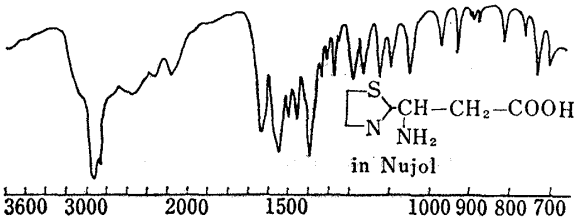

Fig. 5. Infrared Spectrum of 3-(2-Thiazolyl)-β-alanine (in Nujol)

The amino acid was suggested to have L-form concerning with the α-carbon atom by following properties. N-Dithiocarbethoxy derivative[7] of the amino acid shows possitive Cotton effect in the optical rotary dispersion in methanol (Fig. 6), and the amino acid is not decomposed by D-amino acid oxidase derived from hog kidney.

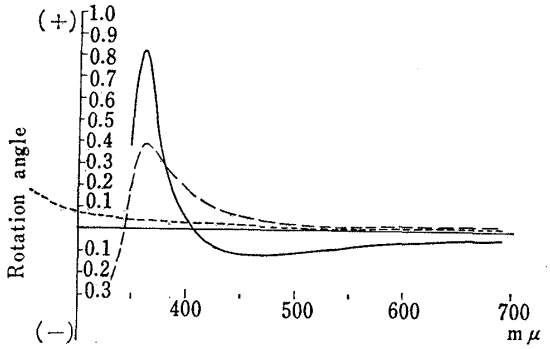

Fig. 6. Optical Rotary Dispersion Curves (Length 0.5 dm.)

----- N-Dithiocarbethoxy-3-methyl-3-phenyl-L-alanine (20 mg./5 ml. MeOH)
——— N-Dithiocarbethoxy-*cis*-3-methyl-L-proline (20 mg./5 ml. MeOH)
------- L-2-Amino-3.3-dimethylbutyric acid HCl (9.8 mg./5 ml. H_2O)

2-Amino-3,3-dimethylbutyric acid,[8] $C_6H_{13}O_2N$ white granules subliming at 210~218°, $[\alpha]_D^{22}$ +4° (c=1, H_2O), is differentiated from leucine, isoleucine and allo-isoleucine on paper chromatograms using several solvents. α-Amino acid is shown by its *pKa* values, 2.4 (COO^-) and 9.7 (NH_3^+). The signals of the nuclear magnetic resonance spectrum (Fig. 7), τ=5.2 (singlet H_2O), τ=6.5 (singlet 1H) and τ=8.92 (singlet 9H), can be explained only by the structure, 2-amino-3,3-dimethylbutyric acid. The amino acid is not oxidized by D-amino acid oxidase. The optical rotary dispersion of the amino acid is the L-amino acid type (Fig. 6).

7) C. Djerassi, H. Wolf, E. Bunnenberg: J. Am. Chem. Soc., **84**, 4552 (1962).
8) F. Knoop, G. Landmann: Zeit. Physiol. Chem., **89**, 157 (1914).

An amino acid which appeared in fractions following L-2-amino-3,3-dimethylbutyric acid was confirmed to be L-valine by the paper chromatography, the infrared spectrum and the optical rotation.

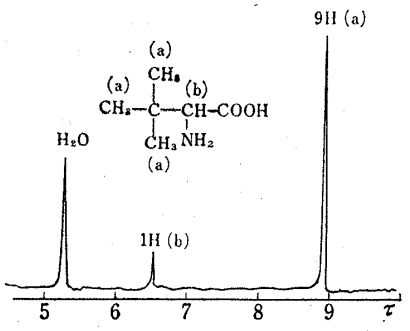

Fig. 7. Nuclear Magnetic Resonance Spectrum of 2-Amino-3,3-dimethylbutyric Acid (in D_2O)

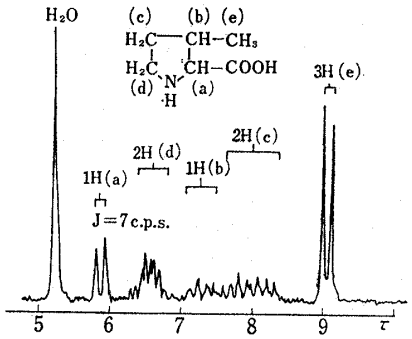

Fig. 8. Nuclear Magnetic Resonance Spectrum of *cis*-3-Methyl-L-proline (in D_2O)

cis-3-Methylproline, $C_6H_{11}O_2N$, has two crystal forms, needless m.p. (decomp.) 218~222° and prisms m.p. (decomp.) 241~244°, $[\alpha]_D^{18}$ −58 (c=1, H_2O). Infrared spectra of the both crystals are shown in Fig. 3 and 4. It is different in Rf values on paper chromatograms from *cis* and *trans*-4-methyl-L-proline.[9] The amino acid gives yellow ninhydrin and blue isatin reactions. The signals τ=5.2 (singlet H_2O), τ=5.9 (doublet 1H, J=7 c.p.s.), τ=6.6 (multiplet 2H), τ=7.4 (multiplet 1H), τ=8.0 (multiplet 2H), and τ=9.05 (doublet 3H) in the nuclear magnetic resonance spectrum (Fig. 8) of the amino acid can be explained only by the structure, 3-methylproline. The coupling constant 7 c.p.s. at τ=5.9 shows the *cis* configration. The amino acid is shown to have L-form by the negative reaction to D-amino acid oxidase and by the positive Cotton effect in optical rotary dispersion (Fig. 6) of the N-dithiocarbethoxyamino acid.

3-(2-Thiazolyl)-β-alanine, $C_6H_8O_2N_2S$ prisms m.p. 200~201° $[\alpha]_D^{18}$ +9° (c=1, H_2O), was identified by paper chromatography comparing with an authentic sample obtained from bottromycin. The infrared spectrum is shown in Fig. 5.

Glycine was identified by paper chromatography and the infrared spectrum.

Experimental

Hydrolysis of Bottromycin A——Bottromycin A (1 g.) was heated in acetic acid anhydride (20 ml.) at 100° for 3 hr. The solvent was evaporated to a small volume and kept in a refrigerator for a night. Crystals of N-(N-acetyl-3-methyl-3-phenylalanyl)-3-(2-thiazolyl)-β-alanine methyl ester were removed by filtration. The filtrate was evaporated to dryness and the residue was refluxed with constant boiling hydrochloric acid (40 ml.) for 24 hr. The hydrolyzate was evaporated to dryness *in vacuo*, dissolved in H_2O and neutalized by addition of IR 4B (OH-type). The reaction mixture was evaporated and separated by a cellulose powder column chromatography (1.6 cm. × 55 cm.) using BuOH-H_2O-AcOH=100:12:100 (upper phase). The eluate was fractionated by 6 ml. by a fraction collector, and each amino acid was separated.

3-Methyl-3-phenyl-L-alanine——The fractions No. 6~7 were combined and evaporated to dryness under vacuum. The residue was recrystallized from EtOH, as white needles 19 mg., m.p. 186~188° (cf. 176~177°).[3] *Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.24; H, 7.23; N, 8.09.

L-2-Amino-3,3-dimethylbutyric Acid——The fractions No. 14~26 were evaporated in vacuum to dryness and the residue dissolved in a small amount of MeOH by warming. After cooled, acetone was added to this solution and kept in a refrigerator to obtain crystals (47 mg.), white granules, subliming at 210~218°. *Anal.* Calcd. for $C_6H_{13}O_2N$: C, 54.94; H, 9.99; N, 10.68. Found: C, 55.01; H, 9.92; N, 10.94.

9) L. F. Burroughs, S. Dalby, G. W. Kenner, R. C. Sheppard: Nature, **189**, 394 (1961).

L-Valine——Valine was obtained by evaporation of the fractions No. 28～34 and washed with MeOH and dried. (38 mg.). $[\alpha]_D^{20}$ +14 (c=1, H_2O), $[\alpha]_D^{20}$ +32 (c=1, *N* HCl).

***cis*-3-Methyl-L-proline**——The amino acid was recovered by evaporation of the fraction No. 35～42 with a small amount of valine and 3-(2-thiazolyl)-β-alanine. The residue was dissolved in a small amount of MeOH to remove the impurities and filtered through active carbon on a funnel with sintered grass to remove the contaminating amino acids. White needles were crystallized by addition of acetone to the MeOH solution, filtered and dried: 18 mg., (Crystal I). The needles shrinked at 190～200° to change plates and decomposed at 218～222°. The filtrate was evaporated to a small volume *in vacuo* and kept in a refrigerator for a night after addition of acetone to obtain further crystals (27 mg., Crystal II): white prisms, converted to plates at 190～200° and decomposed at 241～244°. The identity of the both crystals were shown by the paper chromatography. *Anal.* Calcd. for $C_6H_{11}O_2N$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.35; H, 8.55; N, 11.06.

3-(2-Thiazolyl)-β-alanine——The fractions No. 43～51 were evaporated to dryness and the residue was recrystallized from MeOH to give white prisms (12 mg.), m.p. 200～201° (cf. 197～201.5°).[3] *Anal.* Calcd. for $C_6H_8O_2N_2S$: C, 41.84; H, 4.68; N, 16.27; S, 18.62. Found: C, 41.87; H, 4.74; N, 16.31; S, 18.47.

Glycine——Glycine wasobtained from the fraction No. 98～120.

N-Dithiocarbethoxy-3-methylproline——*N* NaOH (0.2 ml.) and CS_2 (0.007 ml.) were added to an aqueous solution (0.5 ml.) of 3-methylproline (12.9 mg.) in a small flask with a stopper and stirred for 16 hr. by a magnetic stirrer at room temperature. Thereafter, ethyl iodide (0.009 ml.) was added to the reaction mixture and the mixture was further stirred for 8 hr. After the mixture was extracted 3 times with each 5 ml. of ether the reaction mixture was acidified by addition of dil. H_2SO_4 and extracted 3 times with each 5 ml. of ether. N-Dithiocarbethoxy-3-methylproline (20 mg.) was obtained by evaporation of the extracts.

N-Dithiocarbethoxy-3-methyl-3-phenylalanine——3-Methyl-3-phenylalanine (17.9 mg.) was converted to N-Dithiocarbethoxy compound by the same method as that for 3-methylproline.

The authors express their deep gratitude to Prof. S. Yamada and Dr. K. Achiwa of the University of Tokyo for their advices and guidances on the ORD studies. The authors are indebted to Dr. S. Mizushima of this institute for his cooperation to do the enzyme oxidation of the amino acids. The authors also appreciate to Prof. R. C. Sheppard, University of Liverpool, for supplying us *cis* and *trans*-4-methyl-L-proline. Thanks are also obliged to Dr. S. Abe, Toyo Jyozo Co., for the kind supply of the antibiotic No. 3668-L2.

Summary

3-Methyl-3-phenyl-L-alanine, L-2-amino-3,3-dimethylbutyric acid, L-valine, *cis*-3-methyl-L-proline, 3-(2-thiazolyl)-β-alanine and glycine are isolated from hydrochloric acid hydrolyzate of bottromycin A. Characterization and structural studies of the new amino acids, L-2-amino-3,3-dimethylbutyric acid and *cis*-3-methyl-L-proline, are described.

(Received November 18, 1964)