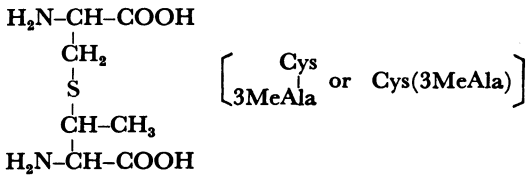
Syntheses of Four Stereoisomers of β-Methyllanthionine.

Tateaki WAKAMIYA, Yoshiaki ODA, Koichi FUKASE, and Tetsuo SHIBA*
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560
(Received September 27, 1984)

Four stereoisomers of β-methyllanthionine were synthesized by coupling of *N*-benzyloxycarbonyl-3-methyl-D-cysteine with β-chloroalanine. ^{1}H NMR analysis and retention time in amino acid analysis were studied on these compounds.

β-Methyllanthionine is a unique sulfide amino acid which was first found as a constituent of peptide antibiotics subtilin[1] and nisin[2] and also occurred in yeast.[3] Recently, the same amino acid was observed in a new angiotensin converting enzyme inhibitor, ancovenin.[4] The configuration of this amino acid in



β-Methyllanthionine = (3-Methylalanin-3-yl)cysteine

all these biologically active peptides was determined to be of (2*R*)-*S*-[(2*S*,3*S*)-3-methylalanin-3-yl]cysteine.[5] To confirm this result synthetically, we prepared four stereoisomers in which the configuration of α-carbon atom of 3-methylalanine part was fixed in *S* configuration corresponding to D-amino acid. Another four possible compounds of *R* configuration on α-carbon atom of 3-methylalanine moiety were not prepared in this work, since all the physicochemical properties except the opposite sign of specific rotation are obtainable from the corresponding enantiomer with (2*S*)-3-methylalanyl configuration. Although Okawa *et al.* reported the syntheses of two enantiomers of protected *threo*-β-methyllanthionine by addition of *N*-benzyloxycarbonyl-L-cysteine benzyl ester to benzyl *N*-benzyloxycarbonyl-(2*S*,3*S*)- or (2*R*,3*R*)-3-methyl-2-aziridinecarboxylate, other enantiomers and free forms of all diastereoisomers have not yet been known in their physical properties.[6]

The synthesis of β-methyllanthionine in this investigation was attempted by coupling of *N*-benzyloxycarbonyl-3-methylcysteine with β-chloroalanine. For this purpose both *threo*- and *erythro*-3-methyl-D-cysteine were required. The *threo* compound was prepared by our previous method *via* an addition of thiocarboxylic acid to aziridinecarboxylic acid derivative obtained from threonine.[7] On the other hand, the *erythro* compound was obtained by S_N2 substitution of *p*-toluenesulfonyloxy group on β-carbon atom of threonine derivative with thiobenzoic acid[8] as shown in Fig. 1. From the amino acid analysis of the product, we could assure that the reaction proceeded without racemization to form *erythro*-3-methyl-D-cysteine, *i.e.*, (2*S*,3*R*)-3-methylcysteine.

Coupling of each *N*-benzyloxycarbonyl-3-methyl-

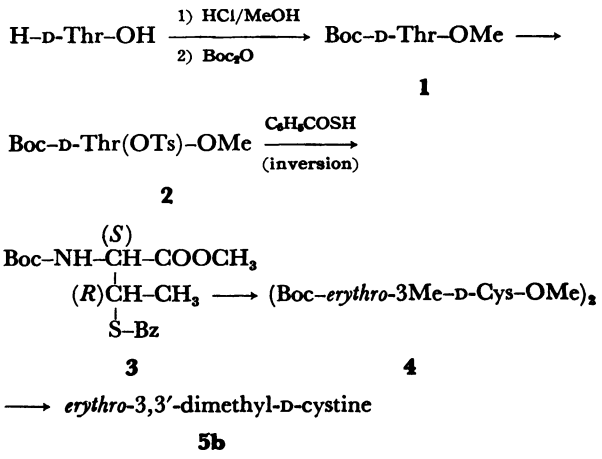


Fig. 1. Preparative scheme of *erythro*-3,3′-dimethyl-D-cystine. Boc=*t*-butoxycarbonyl.

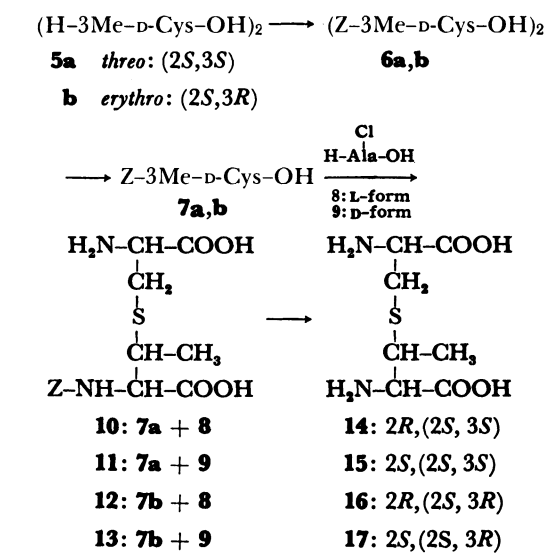


Fig. 2. Preparation of four stereoisomers of β-methyllanthionine.

cysteine (**7a** and **7b**) with L- or D-β-chloroalanine (**8** or **9**) was carried out according to the procedure for the preparation of *meso*-lanthionine by Photaki *et al.*[9] (Fig. 2). Each coupling product, **10**—**13**, was then treated with hydrogen bromide in glacial acetic acid to give corresponding β-methyllanthionine, **14**—**17**, respectively. Physicochemical properties of the compounds **6**—**17** were listed in Table 1. Retention times of final products in amino acid analysis were also given in Table 1. ^{1}H NMR analysis of the compounds **10**—**17** was summarized in Table 2.

TABLE 1. ANALYTICAL DATA OF STEREOISOMERS OF β-METHYLLANTHIONINE AND THE COMPOUNDS RELATED TO THEIR SYNTHESES

Compound	Yield/%	Mp (θ_m/°C, dec)	$[\alpha]_D$/°	Formula	Found (Calcd) (%) C	H	N	S	Retention time in amino acid analysis (min)
6a	96	oil	−28.5(t=32°C)[b]	$C_{24}H_{28}N_2O_8S_2$	53.44 (53.72	5.42 5.26	5.30 5.22	11.93 11.95)	
6b	89	oil	+135(t=19°C)[b]	$C_{24}H_{28}N_2O_8S_2$	53.52	5.14	5.25	11.99	
7a[a]	quant	oil		$C_{12}H_{15}NO_4S$					
7b[a]	quant	oil		$C_{12}H_{15}NO_4S$					
10	53	191—193	−15(t=24°C)[c]	$C_{15}H_{20}N_2O_6S$	50.71 (50.55	5.64 5.66	7.78 7.86	8.98 9.00)	
11	35	204—208	−31(t=24°C)[c]	$C_{15}H_{20}N_2O_6S$	50.41	5.64	7.80	8.81	
12	48	192—195	+46(t=27°C)[c]	$C_{15}H_{20}N_2O_6S$	50.52	5.68	7.84	9.00	
13	53	189—190	−20(t=27°C)[c]	$C_{15}H_{20}N_2O_6S$	50.18	5.65	7.64	8.81	
14	87	228—231	−36(t=23°C)[d]	$C_7H_{14}N_2O_4S$ with $0.6H_2O$ (36.07	36.03 6.57	6.33 12.02	11.91 13.76)	13.71	54.0
15	89	205—206	−48(t=23°C)[d]	$C_7H_{14}N_2O_4S$ with $1.1H_2O$ (34.73	34.53 6.74	6.73 11.57	11.42 13.24)	13.73	54.0
16	quant	258—261	+53(t=23°C)[d]	$C_7H_{14}N_2O_4S$ with $0.6H_2O$ (36.07	36.16 6.57	6.38 12.02	12.16 13.76)	13.33	62.3
17	90	203—226	+5.0(t=27°C)[d]	$C_7H_{14}N_2O_4S$ with $0.9H_2O$ (35.26	35.42 6.68	6.53 11.75	11.68 13.44)	13.41	51.4

a) These compounds were used for the next coupling without purification to avoid reoxidation. b) c 1.18 in ethanol. c) c 0.25 in 1M HCl. d) c 0.22 in 1M HCl. e) Column: Hitachi #2618 resin (one column method, 0.9×25 cm, 55°C), buffer: 0.20 M sodium citrate (pH 3.0).

TABLE 2. CHEMICAL SHIFTS OF THE COMPOUNDS **10**—**17** IN ^{1}H NMR

Compound	Cys part 2-CH	Cys part 3-CH_2	3MeAla part 2-CH	3MeAla part 3-CH	3MeAla part 3-CH_3	Z group —CH_2—	Z group C_6H_5—
10[a]	4.38(dd)	3.26(−)[c]	4.54(d)	3.52(m)	1.37(d)	5.17(s)	7.46(s)
11[a]	4.40(t)	3.30(d)	4.56(d)	3.58(m)	1.41(d)	5.18(s)	7.44(s)
12[a]	4.45(t)	3.29(d)	4.65(d)	3.54(m)	1.30(d)	5.22(s)	7.50(s)
13[a]	4.00(t)	3.53(d)	4.64(d)	3.71(m)	1.40(d)	5.18(s)	7.42(s)
14[b]	3.90(dd)	3.13(−)[c]	3.81(d)	3.49(m)	1.40(d)	—	—
15[b]	3.97(t)	3.17(d)	3.89(d)	3.55(m)	1.46(d)	—	—
16[a]	4.52(t)	3.39(d)	4.45(d)	3.72(m)	1.41(d)	—	—
17[b]	4.05(t)	3.19(d)	3.98(d)	3.57(m)	1.34(d)	—	—

Abbreviations; s: singlet, d: doublet, dd: double doublet, t: triplet, m; multiplet.
a) Measured in 2 M DCl. b) Measured in D_2O. c) Three peaks due to A_2B system were observed.

Of four stereoisomers of β-methyllanthionine obtained, (2*R*)-*S*-[(2*S*,3*S*)-3-methylalanin-3-yl]cysteine (**14**) was identical with the natural one from nisin or ancovenin confirming the assumed structure unequivocally. Now, these synthetic compounds will be useful as authentic samples for structural determination of β-methyllanthionine of unknown configuration when found newly in nature.

Experimental

All melting points are uncorrected. The NMR spectra were obtained with Varian XL-100-15 spectrometer and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the external standard. Chemical shifts were given by δ value (ppm) from DSS. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. Amino acid analysis was carried out with a Hitachi KLA-5 analyzer.

N-t-*Butoxycarbonyl*-D-*threonine Methyl Ester* (*1*). To a solution of D-threonine methyl ester hydrochloride[7a] (12.8 g, 75.6 mmol) in anhydrous methanol (160 ml) were added dropwise triethylamine (11.5 g, 113 mmol) and di-*t*-butyl dicarbonate (18.1 g, 83.2 mmol) under ice cooling. The reaction mixture was stirred overnight at room temperature and then concentrated *in vacuo*.

Ethyl acetate extract from an oily residue obtained was washed successively with water, saturated sodium chloride solution, 10% citric acid solution, saturated sodium hydrogencarbonate solution, and saturated sodium chloride solution. The extract was dried over magnesium sulfate and concentrated *in vacuo* to give **1** as an oily product: yield 15.0 g (85.2%); $[\alpha]_D^{32}$ +7.7° (*c* 1.0, $CHCl_3$). Found: C, 50.10; H, 8.17; N, 5.79%. Calcd for $C_{10}H_{19}NO_5 \cdot 0.3H_2O$[10]: C, 50.33; H, 8.28; N, 5.87%.

N-t-*Butoxycarbonyl*-O-p-*toluenesulfonyl*-D-*threonine Methyl Ester* (*2*). To a solution of **1** (9.70 g, 41.6 mmol) in pyridine (120 ml) was added *p*-toluenesulfonyl chloride (79.4 g, 416 mmol) under cooling in an ice-salt bath. The reaction mixture was stirred for 3 h in the chilled bath and then in an ice bath until the starting material disappeared. The reaction mixture was poured onto crushed ice and extracted with ethyl acetate (1 l×2). The extract was subjected to the general aqueous washing procedure as mentioned above.

Ethyl acetate layer was then dried over magnesium sulfate and concentrated *in vacuo*. An oily residue thus obtained was purified by silica-gel column chromatography. Elution with benzene removed unreacted *p*-toluenesulfonyl chloride. The desired *p*-toluenesulfonate **2** was eluted with ethyl acetate as an oily product: yield 14.9 g (92.5%); $[\alpha]_D^{32}$ −15.5° (*c* 1.39, CH_3OH). Found: C, 52.28; H, 6.62; N, 3.59; S, 8.26%. Calcd for $C_{17}H_{25}NO_7S \cdot 0.2H_2O$[10]: C, 52.22; H, 6.55; N, 3.58; S, 8.20%.

S-Benzoyl-N-t-*butoxycarbonyl*-erythro-*3-methyl*-D-*cysteine Methyl Ester (3).* To a solution of potassium hydroxide (10.5 g, 187 mmol) in methanol (100 ml) was added thiobenzoic acid (25.7 ml, 219 mmol). The mixture was stirred for 5 min at room temperature and then concentrated *in vacuo* to give potassium thiobenzoate which was dried in a vacuum desiccator. To potassium thiobenzoate transferred in a reaction vessel was added the compound **2** (18.4 g, 47.5 mmol) in DMF (61 ml) under nitrogen atmosphere with cooling in an ice bath. The solution was stirred for 64 h at room temperature and the reaction mixture was extracted with ethyl acetate. The extract was washed with saturated sodium chloride, sodium hydrogencarbonate and then sodium chloride solutions. Ethyl acetate layer was dried over magnesium sulfate and concentrated *in vacuo*. An oily residue was column-chromatographed on silica gel (Merck #7734, 7×19 cm). Elution with benzene–ethyl acetate (19:1) gave pure **3** as an oily product: yield 7.59 g (45.2%); $[\alpha]_D^{17}$ −23.7° (*c* 1.65, $CHCl_3$). Found: C, 57.23; H, 6.53; N, 3.94; S, 9.06%. Calcd for $C_{17}H_{23}NO_5S \cdot 0.1H_2O$[10]: C, 57.48; H, 6.58; N, 3.94; S, 9.03%.

N,N′-*Bis*(t-*butoxycarbonyl*)-erythro-*3,3′-dimethyl*-D-*cystine Dimethyl Ester (4).* Debenzoylation and oxidation of the compound **3** (100 mg, 0.28 mmol) were carried out in a similar manner as described in the previous paper.[7] After usual treatment of the reaction mixture as described in preparation of **3**, crude product was purified by column chromatography on silica gel (Merck #7734, 0.8×17 cm). Pure oily material was obtained by an elution with benzene-ethyl acetate (9:1): yield 60 mg (85%); $[\alpha]_D^{17}$ +52.9° (*c* 1.14, $CHCl_3$). Found: C, 48.56; H, 7.35; N, 5.65; S, 12.60%. Calcd for $C_{20}H_{36}N_2O_8S_2$: C, 48.37; H, 7.31; N, 5.64; S, 12.91%.

erythro-*3,3′-Dimethyl*-D-*cystine (5b).* A suspension of **4** (1.57 g, 3.16 mmol) in 6M† HCl (40 ml) was heated for 2 h at 110°C. The hydrolyzate was concentrated *in vacuo* and the residue was dissolved in 50% ethanol (30 ml). The solution was neutralized by an addition of pyridine to give crystalline product which was recrystallized from water and ethanol: yield 722 mg (85.1%); mp 177—183°C (dec); $[\alpha]_D^{19}$ +515° (*c* 1.02, 1 M HCl). Found: C, 35.13; H, 6.15; N, 10.07; S, 23.46%. Calcd for $C_8H_{16}N_2O_4S_2 \cdot 0.3H_2O$[10]: C, 35.10; H, 6.11; N, 10.23; S, 23.42%.

N,N′-*Bis*(*benzyloxycarbonyl*)-threo- *and* erythro-*3,3′-dimethyl*-D-cystine (*6a and 6b*). To a solution of the compound **5a**[7] or **5b** (900 mg, 3.36 mmol) in 1 M sodium hydroxide (13 ml) was added benzyloxycarbonyl chloride (1.1 ml, 7.1 mmol) under ice cooling. The solution was stirred for 2 h at 0°C and then extracted with ether. The aqueous layer acidified with 2 M HCl was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated sodium chloride solution, dried over magnesium sulfate, and concentrated *in vacuo*. Each benzyloxycarbonyl derivative **6a** and **6b** was obtained respectively as an oily substance.

N-*Benzyloxycarbonyl*-threo- *and* erythro-*3-Methyl*-D-*cysteine (7a and 7b).* Disulfide derivative **6a** or **6b** (688 mg, 1.28 mmol) was dissolved in methanol (2.7 ml) and concentrated hydrochloric acid (0.83 ml) was added to the solution under ice cooling. To the solution was added zinc powder by portions and the suspension was stirred in an ice bath until the disulfide disappeared. The reaction mixture was filtered to remove zinc powder and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with saturated sodium chloride solution. Ethyl acetate layer was dried over magnesium sulfate and concentrated *in vacuo* to obtain mercapto derivative **7a** or **7b** respectively as an oily product.

Coupling of Mercapto Derivative with β-Chloroalanine. Mercapto derivative **7a** or **7b** (679 mg, 2.52 mmol) was dissolved in 4.4 M potassium hydroxide (1.5 ml). To the solution warmed at 50°C were added β-chloroalanine hydrochloride (403 mg, 2.52 mmol)[11] and 2.6 M potassium hydroxide (1.9 ml). The solution was warmed at 50°C for 30 min and at room temperature for 44 h under argon atmosphere. The reaction mixture was acidified and extracted with ether. Aqueous layer was concentrated *in vacuo* and the residue dissolved in a small amount of 2 M ammonium hydroxide was applied to HP-20 column (3×16 cm). Elution with water and 50% methanol gave desired product which was crystallized by trituration with methanol and ether.

Analytical sample was obtained by further column chromatography on Amberlite IRCG-50 (H^+ form) using water as an eluent. Pure material was recrystallized from water.

β-Methyllanthionine (14—17). Each *N*-benzyloxycarbonyl-β-methyllanthionine derivative (**10—13**) (340 mg, 0.96 mmol) obtained above was dissolved in 25% HBr in glacial acetic acid (12 ml). To the solution kept at room temperature for 2 h was added ether to precipitate respective β-methyllanthionine as hydrobromide. A solution of the hydrobromide in a small amount of water was neutralized with pyridine. Addition of methanol and ether to the solution gave a free amino acid (**14—17**) respectively which was recrystallized from water and methanol. The data of physical properties of these products (**14—17**) were shown in Table 1 and 2.

The authors are deeply indebted to Ajinomoto Co. Ltd., for the supply of D-threonine.

References

1) a) G. Alderton, *J. Am. Chem. Soc.*, **75**, 2391 (1953); b) E. Gross, H. H. Kiltz, and E. Nebelin, *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 810 (1973).

2) E. Gross and J. L. Morell, *J. Am.Chem. Soc.*, **93**, 4634 (1971).

3) P. F. Downey and S. Black, *J. Biol. Chem.*, **228**, 171 (1957).

4) Y. Kido, T. Hamakado, T. Yoshida, M. Anno, Y. Motoki, T. Wakamiya, and T. Shiba, *J. Antibiot.*, **36**, 1295 (1983).

5) J. R. Knox and P. C. Keck, *Biochem. Biophys. Res. Commun.*, **53**, 567 (1973).

6) K. Nakajima, H. Oda, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **56**, 520 (1983).

7) a) T. Wakamiya, K. Shimbo, T. Shiba, K. Nakajima, M. Neya, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **55**, 3878

†1M=1 mol dm^{-3}.

(1982); b) T. Wakamiya, K. Fukase, K. Shimbo, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **56**, 1559 (1983).

8) The use of thioacetic acid did not give a desirable result.

9) I. Photaki, I. Samouilidis, S. Caranikas, and L. Zervas, *J. Chem. Soc. Perkin I*, **1979**, 2599.

10) Complete removal of water from the oily product was extremely difficult because of very hygroscopic property.

11) a) E. Fischer and K. Raske, *Ber.*, **40**, 3717 (1907); b) G. B. Brown and V. du Vigneaud, *J. Biol. Chem.*, **137**, 611 (1940).