Chem. Pharm. Bull. 26(11)3588—3591(1978)

UDC 547.466.1.04.09 : 576.85.098

Amino Acids and Peptides. III.[1,2] Synthesis of Stereoisomeric Alanine Containing Peptide Derivatives and Their Effects on Germination of *Bacillus thiaminolyticus* Spores. (2)[1]

Yoshio Okada, Shin Iguchi, Miyuki Okinaka, Masami Yagyu, Keiji Sano, and Mieko Otani

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University[3]

(Received May 27, 1978)

Stereoisomeric alanine containing peptides were synthesized to study not only the relationship between the structure of alanine containing peptides and their effects on germination of *Bacillus thiaminolyticus* spores but also their antibacterial activity. The peptides obtained did not show any effect on germination. They did not exhibit any antibacterial activity against *Staphylococcus aureus*, *Sarcina lutea*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Keywords——alanylpeptides; chemical synthesis; effects on germination; *Bacillus thiaminolyticus*; structure-activity; antibacterial activity

A program has been initiated in our laboratory directed to the synthesis of stereoisomeric alanine containing peptide derivatives to study their microbiological activities. In our previous paper,[1] we have shown that L-Ala-L-Ala and L-Ala. Gly (**1**) induce germination of *Bacillus thiaminolyticus* spores and L-Ala-D-Ala and Gly-D-Ala (**2**) inhibit those germination. This investigation describes synthesis of stereoisomeric alanyl-β-alanine (**3a, b**), β-alanyl-

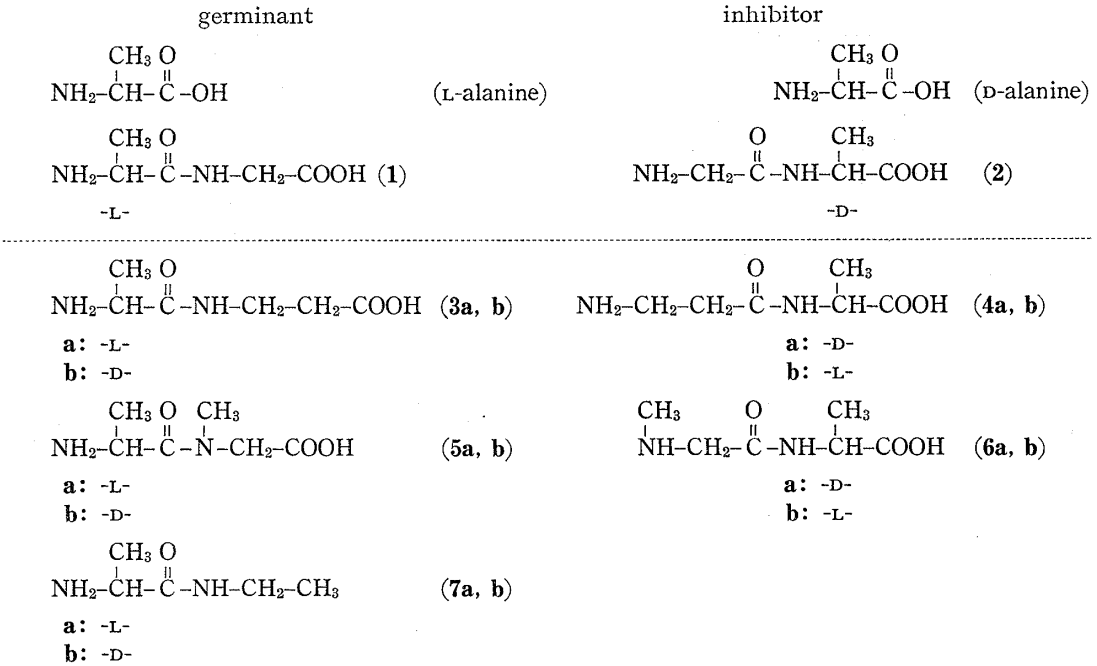

Chart 1

1) Part II: Y. Okada, M. Okinaka, M.Yagyu, K. Watabe, K. Sano, and Y. Kakiuchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 3081 (1976).
2) Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 3485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972).
3) Location: *Ikawadani-machi, Tarumi-ku, Kobe, 673, Japan.*

alanine (**4a,b**), alanylsarcosine (**5a,b**), sarcosylalanine (**6a,b**) and alanine ethylamide (**7a,b**) as shown in Chart 1 and their effect on germination of *Bacillus thiaminolyticus* spores. Furthermore, it deals with the antibacterial activity of these synthetic alanine containing peptides.

Experimental Procedures and Results

Materials and Techniques——Capillary melting points were determined for all peptides and were not corrected (Melting Point Apparatus, Model MP-21, Yamato). Optical rotations were taken with automatic polarimeter (Model DIP-180, Japan Spectroscopic Co. Ltd.). Thin-layer chromatography was performed on silica gel G (Merck). *Rf* values refer to the following solvent systems: Rf_1 *n*-BuOH–AcOH–H_2O (4:1:5), Rf_2 *n*-BuOH–pyridine–AcOH–H_2O (4:1:1:2), Rf_3 AcOEt–$CHCl_3$ (1:2). Amino acid analyses were per-

Table I. Physical Constant and Elemental Analysis of Synthetic Peptides

Compound	mp (°C)	$[\alpha]_D^{27}$	Analysis (%) Calcd. (Found.) C	H	N	Rf_1	Rf_2	Rf_3	Yield (%)
Z-Ala-β-Ala-OMe	92—93	−21.0 (MeOH)	58.4 (58.3	6.54 6.57	9.1 9.1)	0.87	0.86		45.0
-D-	93—94	+19.5	(58.2	6.54	9.2)	0.87	0.89		50.1
Z-β-Ala-Ala-OMe	79—81	−30.1	(58.3	6.60	9.3)	0.91	0.95		58.3
-D-	80—82	+29.0	(58.4	6.57	9.1)	0.89	0.95		61.5
Z-Ala-Sar-OMe[a]	Oil					0.76	0.82	0.55	63.0
-D-	Oil					0.76	0.83	0.54	64.8
Z-Sar-Ala-OMe	Oil					0.76	0.84		40.0
-D-	Oil					0.75	0.84		45.3
Z-Ala-β-Ala-OH	107—109	−19.7 (MeOH)	57.1 (57.2	6.17 6.21	9.5 9.7)	0.86	0.81		53.2
-D-	105—106	+20.0	(57.1	6.16	9.7)	0.86	0.81		51.3
Z-β-Ala-Ala-OH	125—127	−15.2	(57.1	6.15	9.6)	0.87	0.72		57.1
-D-	124—126	+14.5	(57.3	6.16	9.6)	0.86	0.72		82.1
Z-Ala-Sar-OH·DCHA[b]	156—159	−10.9 (H_2O)	65.7 (65.6	8.69 8.83	8.8 8.8)	0.40	0.74		74.4
-D- ·DCHA	157—158	+11.0	(65.7	8.52	8.9)	0.40	0.74		93.0
Z-Sar-Ala-OH	141—145	−22.7 (MeOH)	57.1 (57.1	6.17 6.15	9.5 9.5)	0.84	0.75		56.4
-D-	141—145	+22.5	(57.2	6.15	9.6)	0.85	0.75		51.4
H-Ala-β-Ala-OH	214—216 (dec.)	−16.2 (H_2O)	45.0 (45.2	7.55 7.59	17.5 17.6)	0.16	0.40		68.5
-D-	213—214 (dec.)	+15.2	(44.8	7.59	17.3)	0.16	0.39		70.0
H-β-Ala-Ala-OH	240—241 (dec.)	−32.3	(44.7	7.55	17.3)	0.15	0.36		75.3
-D-	237—239 (dec.)	+35.4	(44.8	7.54	17.4)	0.16	0.38		68.5
H-Ala-Sar-OH·1/4H_2O	148—151 (dec.)	+23.1	43.8 (44.1	7.66 7.56	17.0 17.0)	0.10	0.37		52.9
-D- ·1/4H_2O	151—153 (dec.)	−23.0	(44.1	7.86	17.2)	0.10	0.37		57.6
H-Sar-Ala-OH	180—183 (dec.)	−39.5	45.0 (45.0	7.55 7.21	17.5 17.4)	0.15	0.35		62.4
-D-	182—186 (dec.)	+40.1	(45.2	7.73	17.2)	0.15	0.35		88.8
Z-Ala-NHC_2H_5	126—127	−13.7 (MeOH)	62.4 (62.6	7.25 7.26	11.2 11.1)	0.93	0.87		91.9
-D-	126—127	+12.6	(62.2	7.26	11.2)	0.90	0.89		80.0
H-Ala-NHC_2H_5·HCl	243—244 (dec.)	+13.3 (H_2O)	39.4 (39.3	8.53 8.64	18.4 18.5)	0.39	0.61		51.6
-D- ·HCl	242—244	−12.7	(39.4	8.68	18.6)	0.41	0.61		58.0

a) Purified by column chromatography.
b) Identified as dicyclohexylamine salt.

formed in an amino acid analyzer (Model JLC-6AH, JEOL Co., Ltd.). Silicic acid (Mallinchrodt) was used for column chromatography. *Bacillus thiaminolyticus* MATSUKAWA *et* MISAWA was gift of Professor M. Kondo, Faculty of Pharmaceutical Sciences of Osaka University. Germination was estimated as described previously.[1]

Synthesis of Peptides——L-Ala-β-Ala (**3a**), D-Ala-β-Ala (**3b**), β-Ala-L-Ala (**4a**), β-Ala-D-Ala (**4b**),[4] L-Ala. Sar (**5a**), L-Ala. Sar(**5b**), Sar-L-Ala (**6a**) and Sar-L-Ala (**6b**) were prepared as follows. N^{α}-Benzyloxycarbonyl-amino acid and amino acid methyl ester were coupled with N,N′-dicyclohexylcarbodiimide (DCC)[5] to form N-protected dipeptide methyl ester, which was purified by recrystallization or silica gel column chromatography. Those protected peptides were saponified with 1 N NaOH and hydrogenated over palladium catalyst to afford the corresponding dipeptides, which were purified by recrystallization from H_2O and EtOH. L-Ala-NHC_2H_5·HCl (**7a**)[6] and D-Ala-NHC_2H_5·HCl (**7b**) were prepared by debenzyloxycarbonylation of N^{α}-benzyloxycarbonyl-L- or D-alanine ethylamide in the presence of 1 N HCl which was synthesized by *p*-nitrophenyl ester method[7] from N^{α}-benzyloxycarbonyl-L- or D-alanine and ethylamine respectively and were recrystallized from EtOH. Physical properties and analytical data of the purified peptides and their intermediates are presented in Table I.

Those peptides obtained above were homogeneous and free from constituent amino acids upon thin-layer chromatography and amino acid analyzer. And amino acid analysis of acid hydrolysates of those peptides gave a composition expressed in molar ratios in good agreement with the theoretically expected values. These peptides did not exhibit any effects on germination of *Bacillus thiaminolyticus* spores and did not show any antibacterial activity against gram positive organisms, *Staphylococcus aureus* and *Sarcina lutea*, and gram negative organisms, *Escherichia coli* and *Pseudomonas aeruginosa* in the concentration of 100 μg/ml.

Discussion

L-Alanine is the most effective for germination of *Bacillus* and *Clostridium* species[8] and Kawasaki *et al.*[9] reported that free amino and carboxyl groups and α-hydrogen atom in L-alanine were important for induction of germination. In our previous report[1] it was shown that dipeptides, L-Ala-L-Ala and L-Ala. Gly (**1**) had ability to induce germination of *Bacillus thiaminolyticus* spores and the free amino group of L-alanyl residue and free carboxyl group in the dipeptides are essential for the effectiveness. It was also revealed that eight kinds of stereoisomeric alanyltripeptides did not have any germinability. In this investigation, L-Ala-β-Ala (**3a**) in which peptide chain was longer than L-Ala. Gly (**1**) by CH_2 (*ca.* 1.5Å), L-Ala. Sar (**5a**) which was considered to be methylated on amide nitrogen atom of **1** and L-Ala-NHC_2H_5 (**7a**) which differed from **1** in that the carboxyl group of **1** was replaced with methyl group were synthesized. These peptides did not induce germination of *Bacillus thiaminolyticus* spores. These findings suggest that changes of the chain length and conformation of dipeptides are responsible for the loss of activity and give further support to importance of free carboxyl group in dipeptides to effect on germination.[1] With regard to inhibitor, we reported that besides D-alanine,[10] L-Ala-D-Ala and Gly-D-Ala (**2**) also inhibited L-Ala or L-Ala-L-Ala induced germination of *Bacillus thiaminolyticus* spores and we discussed the relationship between the structure and inhibitory activity.[1] In order to gain further insight about such relationship, we synthesized β-Ala-D-Ala (**4a**) in which the peptide chain was increased by CH_2 (*ca.* 1.5Å) compared with Gly-D-Ala (**2**), and Sar-D-Ala (**6a**) in which amino group was methylated and basic character of this dipeptide was increased as compared with (**2**). We found that these synthetic D-alanine containing dipeptides did not show any inhibitory effect on germination of *Bacillus thiaminolyticus* spores. The loss of inhibitory

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10) C. Kawasaki, M. Kondo, and K. Teshima, *J. Food Hyg. Soc. Japan*, **9**, 201 (1968).

activity might be due to the increment by CH_2 (*ca.* 1.5Å) in chain length and the change from primary amino group to secondary amino group in the dipeptide.

From the results obtained above both about induction and inhibition of germination, we can emphasize that a certain distance between primary amino group and free carboxyl group and conformation of the dipeptide most favourable for binding with the receptor site to induce or inhibit germination are required.

Acknowledgement The authors express their sincere appreciation to Professor H. Yajima of the Faculty of Pharmaceutical Sciences of Kyoto University and Professor M. Kondo of the Faculty of Pharmaceutical Sciences of Osaka University for their encouragement during the course of this investigation. They are grateful to Dr. G.P. Schwartz of the Department of Biochemistry of Mount Sinai School of Medicine of the City University of New York, U.S.A. for his help in preparation of this manuscript. They also thank Mr. O. Wakamatsu and Miss S. Toji for technical assistance.

[Chem. Pharm. Bull. 26(11)3591—3593(1978)]

UDC 546.725.04 : 547.631.107.04

Iron Carbonyls as Mild Friedel-Crafts Catalytic Agent[1]

TAKEHISA KUNIEDA, YUKISHIGE ITO, and TAKEO TAKIZAWA

Faculty of Pharmaceutical Sciences, University of Tokyo[2]

(Received June 2, 1978)

Iron carbonyls may act as an effective agent to moderate the Friedel-Crafts type reaction with allylic polyhalides.

Keywords——iron carbonyl; vinylene carbonate telomer; Friedel-Crafts reaction; allylic halide; coupling reaction

In the course of a study on the synthetic utility of the vinylenecarbonate telomers,[3] mild catalytic activity of diiron enneacarbonyl as well as an effectiveness of iron pentacarbonyl has been recognized in the Friedel-Crafts type alkylation of benzene with the allylic halides **2** and **3** readily derived from the vinylenecarbonate-polyhalomethane adducts **1**.

Scant information is available on catalytic ability of metal carbonyl[4] for the Friedel-Crafts type reaction, though there has been used such a wide variety of catalysts that it is difficult to assess their particular advantages.[5]

Dehydrohalogenation of the bromo (**1**, X=Br) and the chloro (**1**, X=Cl) telomers[6] was performed by the action of triethylamine in benzene to afford the reactive allylic halides **2** and **3**, respectively, as major products, which were sensitive toward nucleophiles such as water and amines to give 2-hydroxy-3, 3-dichloroacrolein and 4-hydroxy-5-dichloromethylene-2-oxazolidone.[7] The former halide **2** underwent smooth allylic rearrangement to the isomer **3**

1) This constitutes part XIII in the series, "Telomers and Oligomers of Vinylene Carbonate." Part XII: Y. Nii, T. Kunieda, and T. Takizawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 1999 (1978).
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