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Amino Acids and Peptides. V.[1,2] Synthesis of N-L- and D-Alanyl-1-aminoethylphosphonic Acids

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N-L-Alanyl-1-aminoethylphosphonic acid (IIIa) and N-D-alanyl-1-aminoethylphosphonic acid (IIIb) were synthesized. IIIb did not exhibit any antibacterial activity against gram-positive or gram-negative organisms, but IIIa showed antibacterial activity against some gram-negative organisms.

Keywords——phosphonic acid derivatives; L-alanylpeptide; D-alanylpeptide; chemical synthesis; antibacterial activity

Some antibiotics, such as penicillins, cephalosporins and D-cycloserine, inhibit the growth of bacteria by interfering with the biosynthesis of peptidoglycan of the bacterial cell walls.[4] It is well known that the structure of D-cycloserine is similar to that of D-alanine[5] and that the highly reactive CO-N bond in the β-lactam ring of penicillin is an analog of the CO-N bond in D-alanyl-D-alanine,[6,7] which is a constituent peptide of peptidoglycan of the bacterial cell walls. The synthesis of stereoisomeric alanine-containing peptide derivatives is under way in our laboratory in order to study their microbiological activity.[8] Previously, Huber *et al.*[9] reported the synthesis of N-D-alanyl-1-aminoethylphosphonic acid (D-Ala-Ala(P)), which is a phosphonic acid analog of D-alanyl-D-alanine and exhibits antibactrial activity against *Pseudomonas aeruginosa*. Recently, Allen *et al.*[10] synthesized L-Ala-L-Ala(P) (named alaphosphin), which, unlike typical β-lactam antibiotics, is more effective against gram-negative than gram-positive organisms. They reported that L-Ala(P) inhibited the biosynthesis of D-Ala-D-Ala within the bacterial cell and that L-Ala(P) had to be combined in a suitable di- or higher peptide to permit its transport into the cell.

In view of these interesting observations, we undertook the synthesis of stereoisomeric alanine-containing phosphonic acid derivatives. The present report describes a convenient synthesis of N-L- and D-alanyl-1-aminoethylphosphonic acids (IIIa and IIIb) as well as their antibacterial activity. The synthetic route to the key intermediate, diethyl 1-aminoethylphosphonate (I) is illustrated in Fig. 1. Huber *et al.*[9] synthesized diethyl 1-aminoethylphosphonate (I) by the benzyloxycarbonylation of 1-aminoethylphosphonic acid[11] followed by esterification with triethyl orthoformate and catalytic hydrogenation. We synthesized I

1) Part IV: Y. Okada, Y. Tsuda and M. Yagyu, *Chem. Pharm. Bull.*, **28**, 310 (1980).
2) Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, 1726 (1972). Z=benzyloxycarbonyl, Ala(P)=1-aminoethylphosphonic acid, L-Ala-L-Ala(P)=(*R*)-1-(L-Alanyl-amino)-ethylphosphonic acid.
3) Location: *Ikawadani-machi, Tarumi-ku, Kobe, 673, Japan.*
4) J.L. Strominger, *K.* Izaki, M. Matsuhashi and D.J. Tipper, *Federation Proc.*, **26**, 9 (1967).
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8) Y. Okada, S. Tani, Y. Yawatari and M. Yagyu, *Chem. Pharm. Bull.*, **24**, 1925 (1976); Y. Okada, M. Okinaka, M. Yagyu. K. Watabe, K. Sano and Y. Kakiuchi, *ibid.*, **24**, 3081 (1976); Y. Okada, S. Iguchi, M. Okinaka, M. Yagyu, K. Sano and M. Otani, *ibid.*, **26**, 3588 (1978).
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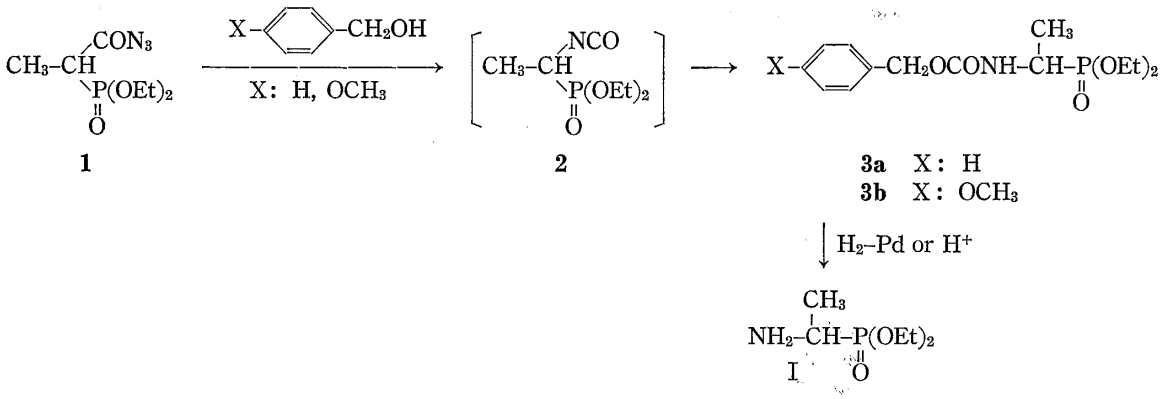
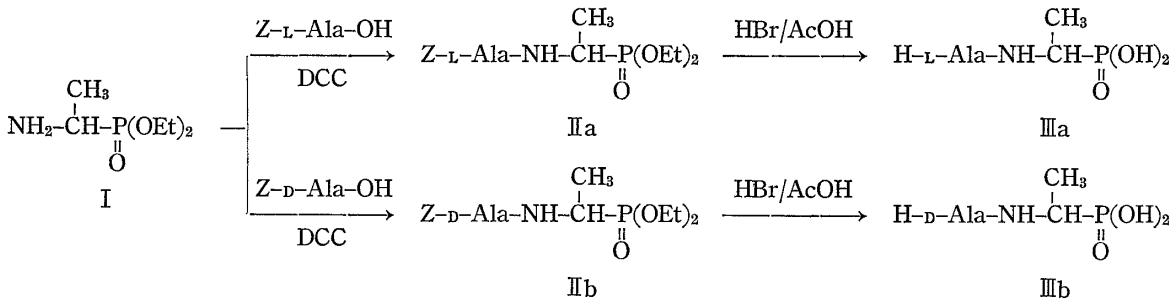


1 2 **3a** X: H **3b** X: OCH_3 I

Fig. 1. Synthetic Route to Diethyl 1-Aminoethylphosphonate I



I IIa IIIa IIb IIIb

Fig. 2. Synthetic Scheme for N–L– and D–Alanyl-1-aminoethylphosphonic Acids

by the route shown in Fig. 1. Diethyl α-phosphonopropionic acid azide (**1**)[11] was combined with benzyl alcohol or *p*-methoxybenzyl alcohol. **1** rearranged to the corresponding isocyanate (**2**), which reacted with the hydroxyl group of the alcohol employed to give diethyl N-benzyloxycarbonyl-1-aminoethylphosphonate (**3a**) or diethyl N-*p*-methoxybenzyloxycarbonyl-1-aminoethylphosphonate (**3b**). The reaction was monitored by following the characteristic IR (infrared spectrum) band (ν CON_3: 2140 and ν NCO: 2275 cm^{-1}). After the disappearance of the absorbances at 2140 and 2275 cm^{-1}, **3a** or **3b** was isolated from the reaction mixture in a crystalline form after purification by column chromatography on silica gel. **3a** or **3b** was converted to the corresponding amine, diethyl 1-aminoethylphosphonate (I) by catalytic hydrogenation over palladium. I was coupled with N-benzyloxycarbonyl-L- or D-alanine by the DCC method to give diethyl N-benzyloxycarbonyl-L-or D-alanyl-1-aminoethylphosphonate (IIa or IIb) as shown in Fig. 2. IIa and IIb were purified by silica gel column chromatography using chloroform. They each exhibited a single spot upon thin-layer chromatography on silica gel. Huber *et al.*[9] treated IIb with HBr/AcOH for 2 hr at room temperature to remove the benzyloxycarbonyl group and the ethyl ester group. However, we found that under these conditions, the ethyl ester group was not completely removed, judging from the results of thin-layer chromatography on silica gel and elemental analysis. We treated IIa and IIb with 25% HBr/AcOH at room temperature for 3 hr. The desired peptides, IIIa and IIIb, were obtained in pure form in the manner described by Huber *et al.*[9] in the synthesis of IIIb. Since racemic I was employed as the starting material, IIIa should be a mixture of diastereoisomers, L-alanyl-L-1-aminoethylphosphonic acid and its L-D isomer and IIIb should consist of D-D and D-L. However, IIIa and IIIb each showed a single spot on thin-layer chromatography and gave a single peak on the amino acid analyzer. Amino acid analysis of the acid hydrolysates of IIIa and IIIb gave molar ratios in a good agreement with the theoretically expected values.

11) J.R. Chamber and A.F. Isbell, *J. Org. Chem.*, **29**, 832 (1964).

Table I. *In Vitro* Antibacterial Spectra of IIIa and IIIb

Organisms	MIC (μg/ml)	
	IIIa	IIIb
E. coli, 1004	50	i
E. coli, 1006	12.5	i
S. flexneri 2a, 5503	25	i
Sal. enteritidis, IID 604	i	i
H. alvei, IID 978	i	i
C. freundii, IID 976	100	i
Pr. inconstans, 403	i	i
K. pneumoniae, 502	i	i
Ent. colacae, 12001	100	i
Ent. cloacae, 12005	100	i
Ent. aerogenes, ATCC 8329	100	i
Ser. marcescens, 13001	100	i
Ser. marcescens, 13028	i	i
Ps. aeruginosa, 2131	i	i
Ps. maltophilia, IID 1275	100	i
S. epidermidis, 7035	i	i
Str. mitis, IID 685	i	i
B. subtilis, ATCC 6633	i	i

MIC=minimum inhibitory concentration in μg/ml, i=MIC> 200 μg/ml.

Finally, the antibacterial activities of the two synthetic peptides (IIIa) and (IIIb) were tested and the antibacterial spectra *in vitro* are shown in Table I. Although IIIb did not exhibit any antibacterial activity against gram-positive or gram-negative organisms at a concentration of 200 μg/ml, IIIa showed antibacterial activity against some gram-negative organisms, as shown in Table I.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-180 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates were determined with a JEOL JLC-6AH amino acid analyzer. NMR spectra were obtained in $CDCl_3$ or D_2O with a 90 MHz (Hitachi R22) instrument. Chemical shifts are given in p.p.m. relative to Me_4Si as an internal standard. Solvents were removed by evaporation *in vacuo* at a bath temperature of 40—50° in a rotary evaporator. For column chromatography, a Toyo SF-160 K fraction collector was used. On thin-layer chromatography (Kieselgel G, Merck), Rf^1, Rf^2 and Rf^3 values refer to the systems of $CHCl_3$, MeOH and H_2O (8: 3: 1), $CHCl_3$, MeOH and AcOH (90: 8: 2) and *n*-butanol, AcOEt, AcOH and H_2O (1: 1: 1: 1), respectively.

Diethyl N-Benzyloxycarbonyl-1-aminoethylphosphonate (3a)——Diethyl-α-phosphonopropionic acid azide was prepared from the corresponding hydrazide (10 g) according to the procedure of Chamber and Isbell.[11] Benzyl alcohol (9.7 g) was added to the ethereal solution of this azide (100 ml). This solution was allowed to stand at room temperature for 4 days. After removal of the solvent, the oily residue was applied to a column of silica gel (3×55 cm), and elution with chloroform (2700—3000 ml) afforded purified **3a**; yield 3.15 g (22.4%). mp 40—42°, Rf^2 0.66, NMR ($CDCl_3$) δ 7.3 (5H, s, aromatic protons), 5.1 (2H, s, $C_6H_5C\underline{H}_2O$), 4.25—3.92 (5H, 2×($POCH_2$) and $CH_3C\underline{H}P$) and 1.49—1.16 (9H, 2×($POCH_2C\underline{H}_3$) and $C\underline{H}_3CHP$); MS *m/e*: 315 (M^+). *Anal.* Calcd for $C_{14}H_{22}NO_5P$: C, 53.3; H, 7.03; N, 4.4. Found: C, 53.5: H, 7.08; N, 4.4.

Diethyl N-*p*-Methoxybenzyloxycarbonyl-1-aminoethylphosphonate (3b)——The title compound was prepared in the manner described above using *p*-methoxybenzyl alcohol instead of benzyl alcohol. After silica gel column chromatography, purified **3b** was obtained; yield 3.98 g (25.9%), mp 55—57°, Rf^2 0.63, NMR ($CDCl_3$) δ 7.38—6.78 (4H, aromatic protons), 5.08 (2H, s, $C_6H_4C\underline{H}_2O$), 4.38—3.82 (5H, 2×($POCH_2$–) and $CH_3C\underline{H}P$), 3.82 (3H, s, CH_3O), 1.55—1.18 (9H, 2×($POCH_2C\underline{H}_3$) and $C\underline{H}_3CHP$), MS *m/e*: 345 (M^+). *Anal.* Calcd for $C_{15}H_{24}NO_6P$: C, 52.2; H, 7.00; N, 4.1. Found: C, 52.2; H, 7.21; N, 4.1.

Diethyl N-Benzyloxycarbonyl-L-alanyl-1-aminoethylphosphonate (IIa)——A solution of diethyl 1-aminoethylphosphonate (I) (prepared from 0.89 g of **3a** by catalytic hydrogenation) and N-benzyloxycarbonyl-L-alanine (0.63 g) in CH_2Cl_2 (10 ml) cooled to 0° was treated with DCC (0.64 g). This reaction mixture was stirred at 4° overnight. After removal of the urea derivative and the solvent, the residue was extracted

with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to give an oily residue. Column chromatography on silica gel (1.3×30 cm) was then carried out. Elution with chloroform (800—1200 ml) provided purified IIa as an oil; yield 0.67 g (61.4%), $[\alpha]_D^{23}$ −9.8° (c=1.0, MeOH), Rf^1 0.63, Rf^2 0.50, NMR ($CDCl_3$) δ 7.3 (5H, s, aromatic protons), 5.1 (2H, s, $C_6H_5C\underline{H}_2O$), 4.69—3.9 (6H, C<u>H</u>CONHC<u>H</u>, 2×($POCH_2$)), 1.5—1.15 (12H, 2×($CHC\underline{H}_3$) and 2×($POCH_2C\underline{H}_3$)), MS m/e: 386 (M^+).

Diethyl N-Benzyloxycarbonyl-D-alanyl-1-aminoethylphosphonate (IIb)——**IIb** was synthesized in the manner described above. A solution of diethyl 1-aminoethylphosphonate (I) (prepared from 0.89 g of **3a** by catalytic hydrogenation) and N-benzyloxycarbonyl-D-alanine (0.63 g) in CH_2Cl_2 (10 ml) cooled to 0° was treated with DCC (0.64 g). After column chromatography on silica gel (1.3×35 cm), purified IIb was obtained as an oily material; yield 0.86 g (78.9%), $[\alpha]_D^{23}$ +12.5° (c=1.0, MeOH), Rf^1 0.63, Rf^2 0.50, NMR ($CDCl_3$) δ 7.3 (5H, s, aromatic protons), 5.1 (2H, s, $C_6H_5C\underline{H}_2O$), 4.69—3.9 (6H, C<u>H</u>CONHC<u>H</u> and 2×($POCH_2$)), 1.53—1.14 (12H, 2×($CHC\underline{H}_3$) and 2×($POCH_2C\underline{H}_3$)), MS m/e: 386 (M^+).

N-L-Alanyl-1-aminoethylphosphonic Acid (IIIa)——IIa (0.67 g) was dissolved in 25% HBr/AcOH (5 ml) and the solution was stirred at room temperature for 3 hr. Addition of ether gave a white precipitate, which was collected by decantation, washed with ether and dried over KOH pellets *in vacuo*. The resulting HBr salt was dissolved in water (8 ml) and treated with Ag_2O (0.25 g) for 30 min. After removal of the precipitate by filtration, the filtrate was passed through an amberlite IRC-50 (H^+ form) column (1×15 cm) and the eluate was concentrated *in vacuo*. EtOH was added to the residue to give IIIa; yield 0.11 g (32.0%), mp 260—265° (dec.), $[\alpha]_D^{25}$ +15.0° (c=0.5, H_2O), Rf^3 0.40, NMR (D_2O) δ 4.65—4.41 (2H, C<u>H</u>CONHC<u>H</u>), 2.03—1.95 (3H, $CHC\underline{H}_3$), 1.89—1.64 (3H, $C\underline{H}_3CHP$). *Anal.* Calcd for $C_5H_{13}N_2O_4P\cdot H_2O$: C, 28.0; H, 7.06; N, 13.1. Found: C, 28.2; H, 6.55; N, 13.3. Amino acid ratios in an acid hydrolysate: Ala 1.00; Ala (P) 0.98 (average recovery 88%).

N-D-Alanyl-1-aminoethylphosphonic Acid (IIIb)——IIb (0.86 g) was converted to the desired compound (IIIb) as described for the synthesis of IIIa, yield 0.40 g (91.3%), mp 267—269° (dec.), $[\alpha]_D^{25}$ −17.5° (c=1.0, H_2O), Rf^3 0.40, NMR (D_2O) δ 4.65—4.41 (2H, C<u>H</u>CONHC<u>H</u>), 2.03—1.95 (3H, $CHC\underline{H}_3$), 1.89—1.64 (3H, $C\underline{H}_3CHP$). *Anal.* Calcd for $C_5H_{13}N_2O_4P\cdot 1/2H_2O$: C, 29.3; H, 6.89; N, 13.7. Found: C, 29.5; H, 7.01; N, 13.8. Amino acid ratios in an acid hydrolysate: Ala 1.00; Ala (P) 0.89 (average recovery 90%).

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