Notes

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Synthetic Inhibitors of Enzymes involved in Peptidoglycan Biosynthesis. I. Preparation of D-Alanine Chloromethylketones

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New chloromethylketone derivatives and other D-alanine-containing compounds related to the peptide moiety of the bacterial peptidoglycan were synthesized in order to search inhibitors of the peptidoglycan transpeptidase.

Keywords——amino acid derivatives; peptide derivatives; chloromethylketone; N-methylamino acid; enzyme inhibitors; peptidoglycan transpeptidase

Halomethylketones derived from amino acids or peptides, which have the structure analogous to the substrates of proteases, are well known to inhibit enzymes selectively and irreversibly.[2] It looks promising to apply this concept to the design of inhibitors of peptidoglycan transpeptidase because the mechanism of the action of this enzyme may be postulated to be analogous to that of serine or thiol proteases.[3] The present report describes the synthesis of D-alanine chloromethylketone derivatives, which have the partial structure of peptidoglycan pentapeptide.[4]

Z-D-Ala-CH_2Cl[5] (**1**) was synthesized by the reaction of diazoketone[6] formed from the mixed anhydride of Z-D-Ala-OH and excess ethereal diazomethane with hydrogen chloride.

Dipeptide chloromethylketone derivatives (**4**—**10**) were synthesized by coupling mixed anhydrides of N-protected amino acids with H-D-Ala-CH_2Cl obtained by the deprotection of **1** with a 25% solution of hydrogen bromide in acetic acid.

Tripeptide chloromethylketones were synthesized as follows: Tripeptide esters (**11**—**13**) prepared from N-protected lysine and HBr·H-D-Ala-D-Ala-OMe by DCC or mixed anhydride method were saponified to the acids (**14**—**16**). Compounds **14** and **16** were converted into chloromethylketone derivatives, **17** and **18**, respectively, *via* mixed anhydrides. In order to suppress racemization, activation of the carboxyl groups of the tripeptides into the mixed anhydrides was carried out in THF or DMF at −20 to −30°, according to the procedure of Anderson *et al.*[7]

Dipeptide chloromethylketones with free amino groups appeared to be unstable. When **7** was treated with a 25% solution of hydrogen bromide in acetic acid in the usual manner

1) Location: *Asahi-cho 3-6-6, Machida-shi, Tokyo.*
2) E. Shaw, "The Enzymes," 3rd ed., Vol. I, ed. by P.D. Boyer, Academic Press, Inc., New York, 1970, p. 91.
3) L. Walls (ed.), "Bacterial Membranes and Walls," Marcel Dekker, Inc., New York, 1973.
4) The main part of this work was presented at the 13th Symposium on Peptide Chemistry, Tokyo, Japan, November, 1975. A new system in which the *in vivo* and *in vitro* formation of cross-links in the peptidoglycan of *Bacillus megaterium* can be compared directly has already been developed in our laboratory [T. Oka, *Antimicrob. Agents Chemother.*, **10**, 579 (1976)]. The enzyme-inhibiting activity of the compounds obtained will be discussed elsewhere.
5) Abbreviations of amino acids and protective groups used in this paper are those recommended by IUPAC–IUB Commission on Biochemical Nomenclature: *Pure Appl. Chem.*, **40**, 317 (1974). CH_2Cl=chloromethyl, THF=tetrahydrofuran, DMF=dimethylformamide, DCC=dicyclohexylcarbodiimide, DCHA=dicyclohexylamine, NMM=N-methylmorpholine.
6) A. Thomson and I.S. Denniss, *Eur. J. Biochem.*, **38**, 1 (1973).
7) G.W. Anderson, J.E. Zimmerman, and F.M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012 (1967).

at room temperature, the crude deblocked product was presumed to be composed of mainly 3-(4′-acetamidobutyl)-5,6-dimethylpyrazin-2-one from high-resolution mass spectral data (M^+ 237.1474; calcd. for $C_{12}H_{19}N_3O_2$, 237.1477) and nuclear magnetic resonance (NMR) data (complete disappearance of the methylene singlet of the chloromethylketone and the doublet of the methyl protons of alanine residue, and appearance of a new singlet at δ 2.20). This presumption is compatible with other investigators' observations.[8] Therefore, we prepared tripeptide chloromethylketones **17** and **18** from N-protected tripeptides **14** and **16**, respectively, instead of coupling deblocked dipeptide chloromethylketones with N-protected amino acids.

N-Methylamino acids are constituents of several naturally occurring peptides of pharmaceutical interest. We synthesized, therefore, Z-D-MeAla-CH_2Cl (**21**) and Z-L-Lys(Ac)-D-

Table I. Yield and Physical Constants of D-Alanine Derivatives

No.	Compound	Yield (%)	mp (°C)	$[\alpha]_D^{25}$(°)	Analysis(%) calcd. (found) C	H	N
1	Z-D-Ala-CH_2Cl	80.4	85— 87	+33.3(c=2.0, MeOH)	56.39 (56.49)	5.52 (5.77)	5.48 (5.87)
2	Z-L-Ala-CH_2Cl[a]	74.2	80— 82	−31.6(c=2.0, MeOH)	56.39 (56.30)	5.52 (5.88)	5.48 (5.21)
3	$ClCH_2CO$-DL-Ala-CH_2Cl[b]	40.5					
4	$ClCH_2CO$-DL-Ala-D-AlaCH_2Cl[b]	22.2					
5	Z-D-Ala-D-Ala-CH_2Cl	91.8	121—122		55.15 (55.37)	5.86 (5.63)	8.58 (8.68)
6	Z-L-Ala-D-Ala-CH_2Cl	61.2	136	+32.8(c=2.0, MeOH)	55.15 (55.21)	5.86 (5.73)	8.58 (8.52)
7	Z-L-Lys(Ac)-D-Ala-CH_2Cl	56.0	145—147	+26.3(c=2.0. MeOH)	56.43 (56.17)	6.63 (6.47)	9.87 (9.47)
8	Ac-L-Lys(Z)-D-Ala-CH_2Cl	41.3	106—111	+22.8(c=2.0, MeOH)	56.43 (56.49)	6.63 (6.67)	9.87 (9.73)
9	Z-L-Lys(Z)-D-Ala-CH_2Cl	60.7	116—120	+22.8(c=0.5, MeOH)	60.29 (60.50)	6.23 (6.16)	8.11 (7.89)
10	Z(OMe)-L-Lys(Z)-D-Ala-CH_2Cl	31.8	127—128	+27.8(c=0.5, DMF)	59.17 (59.71)	6.25 (6.39)	7.67 (7.70)
11	Z-L-Lys(Ac)-(D-Ala)$_2$-OMe	73.2	210—212	+25.0(c=0.5, MeOH)	57.72 (57.65)	7.16 (7.12)	11.71 (11.38)
12	Ac-L-Lys(Z)-(D-Ala)$_2$-OMe	28.8	160—165	+28.2(c=0.5, MeOH)	57.72 (57.98)	7.16 (7.33)	11.71 (11.96)
13	Z-L-Lys(Z)-(D-Ala)$_2$-OMe	81.7	156—160	+21.2(c=0.5, MeOH)	61.04 (61.13)	6.71 (6.78)	9.82 (9.94)
14	Z-L-Lys(Ac)-(D-Ala)$_2$-OH	51.6	200—203	+20.4(c=0.5, MeOH)	56.88 (56.63)	6.94 (6.93)	12.06 (11.79)
15	Ac-L-Lys(Z)-(D-Ala)$_2$-OH	38.3	138—143	+17.6(c=0.5, MeOH)	56.88 (57.25)	6.94 (7.01)	12.06 (12.36)
16	Z-L-Lys(Z)-(D-Ala)$_2$-OH	81.3	164—167	+16.6(c=0.5, MeOH)	60.42 (60.15)	6.52 (6.58)	10.07 (10.01)
17	Z-L-Lys(Ac)-(D-Ala)$_2$-CH_2Cl	32.2	180—183		55.59 (55.37)	6.69 (6.85)	11.27 (11.57)
18	Z-L-Lys(Z)-(D-Ala)$_2$-CH_2Cl[b]	50.1	Oil				
19	Z-D-MeAla-OMe[b]	79.0	Oil				
20	Z-D-MeAla-OH	95.3	60— 62	+29.0(c=2.0, AcOH)	60.75 (60.71)	6.37 (6.32)	5.90 (5.86)
21	Z-D-MeAla-CH_2Cl[b]	72.6	Oil				
22	Z-L-Lys(Ac)-D-MeAla-CH_2Cl[b]	50.8	Oil				

a) Reported[9] mp 87—88°.
b) NMR spectral data are presented in the Experimental Section.

8) E.E. Smissman, A. Terada, and S.E. Antably, *J. Med. Chem.*, **19**, 165 (1976); S. Fittkau, *Prakt. Chem.*, **315**, 1037 (1973).

MeAla-CH_2Cl (**22**). Physical constants and analytical data of the compounds obtained in the present study are shown in Table I.

Experimental

All melting points were measured by the capillary method and are uncorrected. Optical rotations were determined in an automatic polarimeter, Hitachi Perkin-Elmer 141. Infrared (IR) spectra were taken on a Hitachi 215 spectrophotometer, NMR spectra at 60 MHz on a Varian T-60 spectrometer using tetramethylsilane as internal standard, and mass spectra on a JEOL JMS-01SG-2 spectrometer. Thin-layer chromatography (TLC) was performed upon Merck silica gel G precoated plates by the ascending method with a solvent system of $CHCl_3$–AcOH (95: 5).

Amino Acid Chloromethylketones[9] (**1—3**)——General Procedure: N-Protected amino acid (10 mmol) was dissolved in THF (50 ml) and the solution was cooled to −30°. NMM (10 mmol) and ethyl chloroformate (10 mmol) were added and the mixture was stirred for 2—5 min. Excess CH_2N_2–ether was added and stirring was continued for 1 hr at 0°. The IR spectrum of the reaction mixture had a strong band at 2100 cm^{-1}, indicative of the diazo stretching frequency. The yellow solution was decolorized with dry HCl. The solution was filtered and the filtrate was evaporated to dryness under vacuum. The residue was redissolved in AcOEt. The solution was washed with H_2O and 1 N $NaHCO_3$, and dried over $MgSO_4$. Evaporation of the solvent gave a yellowish solid which was recrystallized from AcOEt–hexane.

The product (**3**) from $ClCH_2CO$-DL-Ala-OH[10] was a brownish solid and could not be crystallized; NMR (CD_3OD) δ: 1.45 (3H, doublet, J=7 Hz, α-CH_3), 4.20 (2H, singlet, N–$COCH_2Cl$), 4.47 (2H, singlet, C–$COCH_2Cl$).

Dipeptide Chloromethylketones (**4—10**)——General Procedure: The mixed anhydride of N-protected amino acid[11] (10 mmol) was prepared at −30° in THF, as above. The compound **1** (10 mmol) was treated with 25% HBr/AcOH (20 ml) and this deblocked product was added to the mixed anhydride solution without purification. After addition of NMM (10 mmol), the mixture was stirred for 2—3 hr at room temperature. The mixture was filtered, the filtrate was evaporated *in vacuo*, and the residue was dissolved in AcOEt. The solution was washed consecutively with H_2O, 1 N HCl, and 1 N $NaHCO_3$. After drying over $MgSO_4$ and evaporation, recrystallization from AcOEt–hexane gave **5—10**. The compound **4** could not be crystallized; NMR (CD_3OD) δ: 1.40 (6H, 2 doublets, J=7 Hz, 2×CH_3), 4.10 (2H, singlet, N–$COCH_2Cl$), 4.47 (2H, singlet, C–$COCH_2Cl$).

Z-L-Lys(Ac)-D-Ala-D-Ala-OMe (**11**) was prepared from Z-L-Lys(Ac)-OH·DCHA (8.87 g) and HBr·H-D-Ala-D-Ala-OMe (**23**)[12] (4.49 g) by the DCC method using CH_2Cl_2 (100 ml) as the solvent. The products were purified by recrystallization from AcOEt–hexane.

Ac-L-Lys(Z)-D-Ala-D-Ala-OMe (**12**) and Z-L-Lys(Z)-D-Ala-D-Ala-OMe (**13**) were prepared from the corresponding N-protected lysine and **23** by the mixed anhydride method, using THF as the solvent.

Z-L-Lys(Ac)-D-Ala-D-Ala-CH_2Cl (**17**) was prepared as described for **1—3** in DMF (20 ml) from Z-L-Lys-(Ac)-D-Ala-D-Ala-OH (**14**) (465 mg) obtained by saponification of **11**.

Similarly, chloromethylketone **18** was prepared using THF as the solvent, which was obtained as an oil. NMR (CD_3OD) δ: 1.20—2.00 (12H, 2 doublets, J=7 Hz, 2×CH_3 and multiplet, C-$(CH_2)_3$–C), 3.10 (2H, multiplet, N–CH_2), 4.43 (2H, singlet, CH_2Cl), 5.13 (4H, singlet, 2×benzyl), 7.40 (10H, singlet, 2×phenyl).

Z-D-MeAla-OMe (**19**) was prepared from Z-D-Ala-OH (3.0 g) using MeI (7 ml) and Ag_2O (12.5 g) in DMF (40 ml)[13] ; The product was obtained as an oil. TLC: Rf 0.70; NMR ($CDCl_3$) δ: 1.43 (3H, doublet, J=8 Hz, α-CH_3), 2.90 (3H, singlet, N–CH_3), 3.70 (3H, singlet, O–CH_3), 4.5—5.0 (1H, multiplet, α-H), 5.16 (2H, singlet, benzyl), 7.40 (5H, singlet, phenyl).

Z-D-MeAla-CH_2Cl (**21**) was prepared from Z-D-MeAla-OH (**20**) obtained by saponification of **19** in the same manner as described for **1—3**; The product was obtained as an oil. TLC: Rf 0.65; NMR (CD_3OD) δ: 1.33 (3H, doublet, J=7 Hz, α-CH_3), 2.91 (3H, singlet, N–CH_3), 4.30 (2H, singlet, CH_2Cl), 4.5—4.8 (1H, multiplet, α-H), 5.17 (2H, singlet, benzyl), 7.40 (5H, singlet, phenyl).

Debenzyloxycarbonylation of **21** with 25% HBr/AcOH gave HBr..H-D-MeAla-CH_2Cl as a yellow oil, which was chromatographically homogeneous and was used directly for the preparation of **22**.

Z-L-Lys(Ac)-D-MeAla-CH_2Cl (**22**) was prepared in the same manner as **7** and obtained as an oil. TLC: Rf 0.50; NMR ($CDCl_3$) δ: 2.90 (3H, singlet, N–CH_3), 4.24 (2H, singlet, CH_2Cl).

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