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Dehydrooligopeptides. XX. Unusual Peptide Bond Cleavage of Dehydrotripeptide Esters Containing α-Dehydroamino Acid Residue at P_2 by Using Papain[1)]

Chung-gi Shin,* Kazushige Arai, Keitaro Hotta, and Takeshi Kakusho

Laboratory of Organic Chemistry, Faculty of Technology, Kanagawa University, Rokkakubashi, Kanagawa-ku, Yokohama 221

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Enzymatic ester hydrolysis and coupling of various *N*-protected Δ^2-dehydrotripeptide methyl esters (Boc–AA–ΔAA–AA–OMe) (**4**) by using protease papain in McIlvaine buffer are mainly described. The substrates (**4**) used were prepared by one-pot coupling of *N*-carboxy-α-dehydroamino acid anhydride (ΔAA·NCA) with *N*- and *C*-component L-α-amino acids (AA). Even in the enzymatic reaction of **4** containing an unusual ΔAA residue, the normal ester hydrolysis took place to give Boc–AA–ΔAA–AA–OH (**6**) and, in certain cases, the interesting unusual peptide bond cleavage at P_2 of **6** occured further to give the unexpected *N*-(1,2-dioxoalkyl)–AA–OH. Besides examining in detail the differences between the enzymatic actions to the structures of **4**, we also studied the mechanisms of the ester and peptide bond hydrolyses. As the results, the reverse enzymatic coupling of **4** with H–AA–ΔVal–OMe was first achieved to give dehydropentapeptide containing two ΔAA residues.

Recently,[2—6)] we have reported on the selective enzymatic hydrolyses of α-dehydroamino acid (DHA, ΔAA) esters (**1**) and Δ^1-dehydrodipeptide esters (**2**)[7)] by the catalytic action of thiol protease papain (EC 3.4.22.2). In addition, the effective peptide formations by the reverse enzymatic coupling of **1** or **2** with L-α-amino acid anilide and Δ^2-dehydrodipeptide ester,[7)] respectively, have been also attained.

So far, a great number of enzymatic reactions, such as, the optical resolution[8)] and condensation[9)] of an appropriate uncommon α- or β-amino acid derivatives by using various proteases, have been reported. However, there has been no report on the enzymatic hydrolysis and peptide bond formation of a variety of dehydrooligopeptide esters containing one or more unusual ΔAA residues.

In general, in the cases of the enzymatic ester hydrolysis and peptide bond formation of appropriate peptide esters, it is well-known that proteolytic papain requires only a neutral and large proteinic L-α-amino acid (AA) residue, such as phenylalanine (Phe), leucine (Leu), or valine (Val), at P_2,[10)] as illustrated in Fig. 1.

In connection with an extensive examination of the enzymatic dehydropeptide synthesis and the further development of a new catalytic action of cheaper and readily accessible papain in the organic synthesis, Δ^2-dehydrotripeptide methyl esters (**4**) and Δ^2-dehydrodipeptide methyl ester (**5**) were chosen as the substrates. Various *N*-*t*-butoxycarbonyl (Boc)–(*Z*)-Δ^2-dehydrotripeptide methyl esters (**4**: Boc–AA–ΔAA–AA–OMe) were prepared by the condensation of *Z*-form of *N*-carboxy-α-dehydroamino acid anhydride (**3**: ΔAA·NCA: ΔAA: **a**; ΔLeu, **b**; ΔVal, **c**; ΔPhe) with a carboxyl (*C*-) component Boc–L-AA–OH (AA: Gly, Ala, Val, Leu, Phe) and then with an amine (*N*-) component H–L-AA–OMe (AA: Ala, Val, Leu) in one pot, according to the method reported earlier[11—13)] (Scheme 1). On the other hand, Boc–L-AA–ΔVal–OMe has been also already obtained by the one-pot coupling of ΔVal·NCA (**3b**) with Boc–AA–OH (AA: Ala, Val, Leu, Ile, Phe) in MeOH[11—13)] (Scheme 1). Then, thus obtained **4** was subjected to the enzymatic ester hydrolysis. As a result, besides giving the normal hydrolyzate Boc–AA–ΔAA–AA–OH (**6**), according to the sort of ΔAA residue, the **6** as an intermediate was found to further hydrolyze at P_2 to give the unexpected *N*-(4-methyl-1, 2-dioxopentyl) (Mdop)–AA–OH (**7**). The reactive distinctions in the enzymatic actions between the two types of the structures in **4a—q** were examined and then two hydrolytic mechanisms were also studied. Moreover, in the case of **6** as a stable form, the coupling of **4** with HCl·H–AA–ΔVal–OMe (**5**), derived by the deprotection of Boc–AA–ΔVal–OMe with HCl in EtOAc, proceeded smoothly to give $\Delta^{2,5}$-dehydropentapeptide methyl esters (**8**—**11**)[7)] (Scheme 6).

Consequently, herein we wish to report the enzymatic hy-

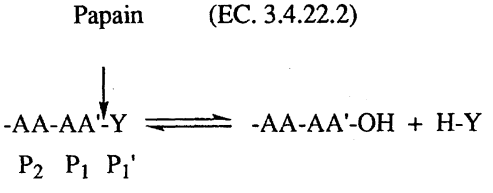
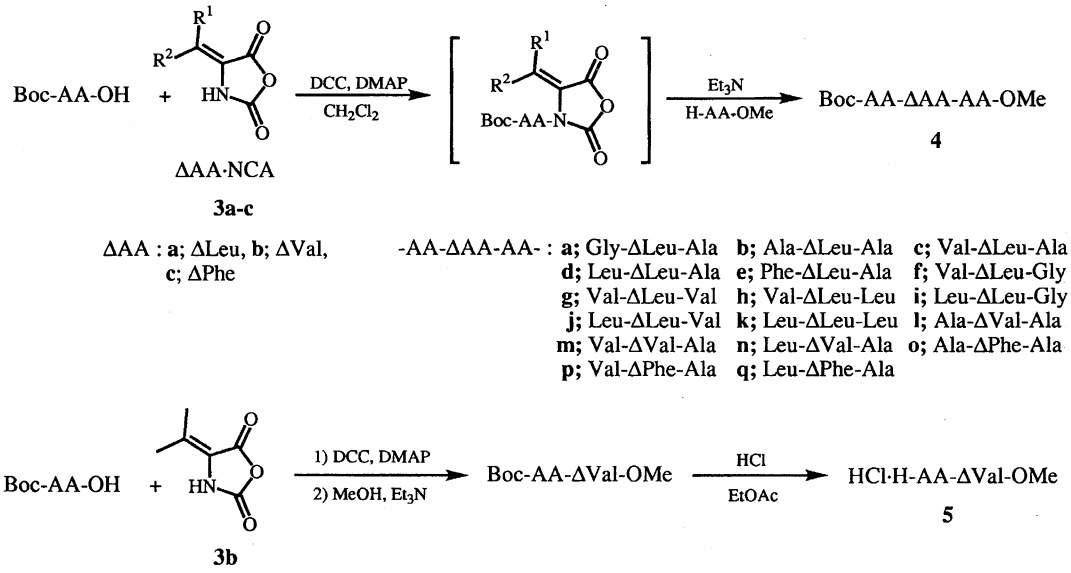


Fig. 1.



Scheme 1.

drolysis of **4** and peptide bond formation by coupling of **4** with **5**. In addition, the formation mechanism of **7** was also examined.

Experimental

General. The melting points were determined with a Yamato Mp-21 micro melting-point apparatus, and were uncorrected. The IR spectra were recorded with a Hitachi 270-30 spectrometer in KBr. The ^{1}H NMR spectra were measured with a JEOL EX 90 spectrometer in a $CDCl_3$ solution with tetramethylsilane used as the internal standard. The specific rotations were measured in a 0.5 dm tube using a JASCO DIP-4 polarimeter in MeOH (Japan Spectroscopic Co., Ltd.). High performance liquid chromatography (HPLC) analyses and separations with a Hitachi 638-50 liquid chromatograph apparatus were performed on an SLE-04H (40×250 mm) column using LiChroSorb RP-18.

Enzyme. Papain (2.8 unit mg^{-1}, crude powder, P3375), purchased from Sigma Chemical Co., U.S.A., was used without further purification.

Boc–AA–ΔAA–AA–OMe (4) as the *C*-Component. To a solution of **3** (5 mmol) and Boc–AA–OH (5.5 mmol) in CH_2Cl_2 (20 ml) was added dicyclohexylcarbodiimide (DCC) (5.5 mmol), with stirring, at −10 °C. After stirring for 20 min, 4-(dimethylamino)pyridine (DMAP) (0.5 mmol) was added to the resultant solution and then stirred for 3 h. To the resulting solution was added H–AA–OMe (6.0 mmol) and triethylamine (TEA) (7.5 mmol), with stirring, at room temperature. After begin stirred overnight, the reaction mixture was concentrated in vacuo to give a residual solid, which was dissolved in ethyl acetate (50 ml). Dicyclohexylurea deposited was filtered off and the filtrate was washed with 1 M HCl (30 ml×2), brine (30 ml×2), and then dried over anhydrous $MgSO_4$. Evaporation in vacuo gave a syrupy residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (1 : 4 v/v) to give crude crystals. Recrystallization from hexane–EtOAc gave **4** as colorless needles. The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **4** are summarized in Tables 1 and 2.

Enzymatic Hydrolyses of 4. Typical Procedure: A suspension (2.5 ml) of an appropriate **4** [200 mM (1 M=1 mol dm^{-3})]

Table 1. The Yields and Melting Points of **4**

Compd	Yield	Mp[a]	Formula	Found (%)		(Calcd)
No.	%	θ_m/°C		C	H	N
4a	62	136—138	$C_{17}H_{29}N_3O_6$	54.97	7.60	11.36
				(54.97	7.87	11.31)
4b	77	85—87	$C_{18}H_{31}N_3O_6$	55.86	8.05	10.81
				(56.09	8.11	10.90)
4c	64	152—153	$C_{20}H_{35}N_3O_6$	57.98	8.48	10.17
				(58.09	8.53	10.16)
4d	65	150—151	$C_{21}H_{37}N_3O_6$	58.88	8.46	9.88
				(59.00	8.72	9.83)
4e	64	146—147	$C_{24}H_{35}N_3O_6$	62.65	7.72	9.18
				(62.45	7.64	9.10)
4f	66	124—125	$C_{19}H_{33}N_3O_6$	57.00	8.15	10.60
				(57.13	8.33	10.52)
4g	53	169—171	$C_{22}H_{39}N_3O_6$	59.60	9.01	9.51
				(59.84	8.90	9.52)
4h	60	111—112	$C_{23}H_{41}N_3O_6$	60.33	9.34	9.52
				(60.64	9.07	9.22)
4i	61	127—129	$C_{20}H_{35}N_3O_6$	57.83	8.38	9.97
				(58.09	8.53	10.16)
4j	70	140—142	$C_{23}H_{41}N_3O_6$	60.83	8.91	9.25
				(60.64	9.07	9.22)
4k	53	129—130	$C_{24}H_{43}N_3O_6$	61.80	9.22	9.14
				(61.38	9.23	8.95)
4l	60	147—148	$C_{17}H_{29}N_3O_6$	54.77	7.65	11.32
				(54.97	7.87	11.31)
4m	57	145—147	$C_{19}H_{33}N_3O_6$	57.37	8.35	10.75
				(57.13	8.33	10.52)
4n	56	170—171	$C_{20}H_{35}N_3O_6$	57.64	8.36	10.51
				(58.09	8.53	10.16)
4o	66	130—132	$C_{21}H_{29}N_3O_6$	59.44	7.14	9.89
				(59.28	7.03	9.88)
4p	70	166—168	$C_{23}H_{33}N_3O_6$	61.86	7.49	9.56
				(61.73	7.43	9.39)
4q	72	138—139	$C_{24}H_{35}N_3O_6$	62.33	7.59	9.19
				(62.45	7.64	9.10)

a) Colorless needles from EtOAc–hexane.

Table 2. The Spectral and Optical Data of **4**

Compd No.	IR, ν/cm^{-1} –NH–	–CONH–	–C=C–	^{1}H NMR, δ α'-H (P_3)	–CH= (*J*/Hz)	α-H (P_1)	$[\alpha]_D^{25}$/° (*c* 1.0, in MeOH)
4a	3388	1686	1629	3.85d	6.37d	4.58dq	−35.0
		1542		(5.7)	(10.1)	(7.3,7.0)	
4b	3394	1752	1629	4,15dq	6.42d	4.60dq	−20.7
		1539		(6.8,6.4)	(10.1)	(7.3,7.0)	
4c	3334	1722	1641	3.89dd	6.42d	4.61dq	−28.5
		1536		(6.8,6.6)	(10.1)	(7.0,7.3)	
4d	3334	1722	1647	4.10m	6.45d	4.60dq	−23.5
		1539			(10.1)	(7.3,7.0)	
4e	3346	1746	1646	4.30dt	6.43d	4.59dq	−19.3
		1533		(7.0,7.5)	(10.1)	(7.3,7.3)	
4f	3322	1746	1650	4.05dd	6.51d	3.84d	−38.2 (*c* = 1.1)
		1524		(3.1,3.1)	(10.1)	(5.7)	
4g	3334	1725	1629	3.96dd	6.36d	4.58dd	−30.8
		1515		(6.2,6.4)	(10.1)	(5.1,5.3)	
4h	3310	1689	1629	3.90dd	6.41d	4.68m	−36.3 (*c* = 1.1)
		1536		(6.8,7.0)	(10.1)		
4i	3304	1704	1644	4.05m	6.54d	4.05m	−38.4
		1506			(10.1)		
4j	3364	1692	1626	4.12m	6.36d	4.55dd	−56.6
		1584			(10.1)	(5.1,5.3)	
4k	3358	1695	1629	4.10m	6.44d	4.67m	−57.5
		1539			(10.3)		
4l	3250	1722	1650	4.11dq	7.15—7.38m	4.49dq	+13.0
		1557		(6.6,7.0)	(+Ph)	(7.3,7.0)	
4m	3256	1719	1644	3.93dd	—	4.58dq	−23.6
		1551		(6.4,6.4)		(7.3,7.3)	
4n	3280	1706	1642	4.05m	—	4.57dq	−17.5 (*c* = 0.1)
		1512				(7.3,7.3)	
4o	3364	1746	1644	4.18dq	7.17—7.44m	4.63dq	−38.6
		1533		(6.8,6.4)	(+Ph)	(7.0,7.3)	
4p	3334	1722	1635	4.00dd	7.21—7.38m	4.66dq	+23.8
		1542		(6.0,6.0)	(+Ph)	(7.3,7.0)	
4q	3334	1722	1647	4.15m	—	4.65dq	−20.8
		1557				(7.0,7.3)	

and papain (30 g dm^{-3}) in the presence of 2-mercaptoethanol (0.1 ml) in McIlvaine buffer was incubated, with shaking, at pH 8.0 and at 35 °C for 24 h. The reaction mixture was diluted with water (20 ml) and then acidified with 1 M HCl. The resulting solution was extracted three times with EtOAc (10 ml×3) and the combined extracts were washed with brine (30 ml) and dried over anhydrous $MgSO_4$. Concentration in vacuo gave a crude syrup or crystals, which were purified by the HPLC method using a mixture of MeOH and water (7 : 3 v/v) to give colorless crystals. Recrystallization from EtOAc–hexane gave Boc–(*Z*)-Δ^2-dehydrotripeptide-OH (**6**: Boc–AA–ΔAA–AA–OH) as colorless needles or *N*-(4-methyl-1,2-dioxopentyl)-L-amino acid (**7**: *N*-Mdop–AA–OH) as colorless needles or syrup. The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **6** and **7** are summarized in Tables 3, 4, 5, and 6.

Coupling of H–Ala–OH with 4-Methyl-2-oxopentanoic Acid. *N*-Mdop–Ala–OMe. To a solution of 4-methyl-2-oxopentanoic acid (0.5 g, 3.8 mmol) in CH_2Cl_2 (10 ml) was added, with stirring, DCC (1.2 g, 5.7 mmol) at −10 °C for 15 min. After stirring for 30 min, a mixture of HCl·H–Ala–OMe (0.6 g, 4.2 mmol) and Et_3N (0.58 ml) was added to the resulting solution, which was then stirred at −10 °C for 30 min and continuously at room temperature for 24 h. After filtering off the deposited dicyclohexylurea, the filtrate was concentrated in vacuo to give a residual syrup. The residue was dissolved in EtOAc (30 ml) and the resulting solution was washed twice with 10% citric acid, twice with saturated aqueous $NaHCO_3$ solution (10 ml) and then brine (10 ml), and finally dried over anhydrous Na_2SO_4. Concentration in vacuo gave a yellow syrup, which was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (80 : 1 v/v) to give *N*-Mdop–Ala–OMe as a colorless syrup. Yield 70% (0.58 g). $[\alpha]_D^{25}$ −41.1 ° (*c* 0.91, MeOH). IR 1746, 1686 cm^{-1}. ^{1}H NMR δ = 0.93 (d, 6H, 2×Me, *J* = 6.6 Hz), 1.48 (d, 3H, Me in Ala, *J* = 7.3 Hz), 2.18 (m, 2H, CH_2, *J* = 6.8 Hz), 3.78 (s, 3H, OMe), 4.55 (dq, 1H, α-H, *J* = 7.3 and 7.5 Hz), 7.42 (br s, 1H, NH). Found: C, 55.59, H; 7.82, N; 6.65%. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51%.

***N*-Mdop–Ala–OH.** Similarly to the case of **4**, the enzymatic hydrolysis of *N*-Mdop–Ala–OMe (108 mg, 0.5 mmol) with papain (75 mg) in the presence of 2-mercaptoethanol (0.1 ml) in McIlvaine buffer (2.5 ml) at pH 8.0 and at 35 °C for 24 h gave *N*-

Table 3. The Yields and Melting Points of **6**

Compd No.	Yield %	Mp[a] θ_m/°C	Formula	Found (%) C	H	(Calcd) N
6c	Quant.	194—195	$C_{19}H_{33}N_3O_6$	57.07	8.32	10.35
				(57.13	8.33	10.52)
6f	65	192 (decomp)	$C_{18}H_{31}N_3O_6$	56.24	8.23	10.51
				(56.09	8.11	10.90)
6g	36	89—90	$C_{21}H_{37}N_3O_6$	59.39	8.95	10.20
				(59.00	8.72	9.83)
6h	27	97—99	$C_{22}H_{39}N_3O_6$	59.86	9.15	9.14
				(59.84	8.90	9.52)
6l	33	104—106	$C_{16}H_{27}N_3O_6$	54.02	7.71	11.51
				(53.77	7.61	11.76)
6m	58	123—125	$C_{18}H_{31}N_3O_6$	56.17	8.34	11.08
				(56.09	8.11	10.90)
6n	79	80—82	$C_{19}H_{33}N_3O_6$	56.50	8.38	10.84
				(56.49	8.36	10.40)
6o	78	151—153	$C_{20}H_{27}N_3O_6$	59.02	6.88	10.57
				(59.25	6.71	10.36)
6p	28	199—201	$C_{22}H_{31}N_3O_6$	61.16	7.17	9.45
				(60.95	7.21	9.69)
6q	70	141—143	$C_{23}H_{33}N_3O_6$	61.49	7.42	9.14
				(61.73	7.43	9.39)

a) Colorless needles from EtOAc–hexane.

Mdop–Ala–OH (**7a**) as a colorless powder. Yield 98% (99 mg).

Reaction of 3a with H–Ala–OMe. To a solution of **3a** (0.40 g, 2.6 mmol) and HCl·H–Ala–OMe (0.44 g, 3.1 mmol) in CH_2Cl_2 (20 ml) was added Et_3N (0.4 ml), with stirring, at −10 °C. After being stirred at room temperature for 8h, the reaction mixture was blended with water (40 ml) and then stirred continuously for 12 h. CH_2Cl_2 (20 ml) was further added to the resulting solution, which was then washed twice with 10% citric acid (10 ml), twice with saturated $NaHCO_3$ aqueous solution (10 ml), brine (10 ml), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residual syrup, which was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (80 : 1 v/v) to give *N*-Mdop–Ala–OMe as colorless syrup. Yield 62%. $[\alpha]_D^{25}$ −41.1° (*c* 0.9, MeOH). IR 1746, 1686 cm^{-1}. ^{1}H NMR δ = 0.93 (d, 6H, Me, *J* = 6.6 Hz), 1.48 (d,

Table 5. The Yields and Physical Melting Points of **7**

Compd No.	Yield %	Mp[a] θ_m/°C	Formula	Found (%) C	H	(Calcd) N
7a	Quant.	121—123	$C_9H_{15}NO_4$	53.36	7.55	6.63
				(53.72	7.51	6.96)
7b	75	Syrup	$C_8H_{13}NO_4$	51.50	6.90	7.76
				(51.33	7.00	7.48)
7c	40	72—74	$C_{11}H_{19}NO_4$	57.32	8.31	5.93
				(57.63	8.35	6.11)
7d	26	Syrup	$C_{12}H_{21}NO_4$	59.52	8.44	5.90
				(59.24	8.70	5.76)

a) Colorless needles from EtOAc–hexane.

Table 6. The Spectral and Optical Data of **7**

Compd No.	IR, ν/cm^{-1} –COOH	–CONH–	–C=O	^{1}H NMR, δ CONH	–CH_2– (*J*/Hz)		$[\alpha]_D^{25}$/° (in MeOH)
7a	1731	1690	1650	7.41d	2.73d		−9.0 (*c* = 0.4)
		1551		(7.7)	(6.8)		
7b	1720	1695	1665	7.38bs	2.73d		—
		1530			(6.8)		
7c	1722	1695	1659	7.34d	2.73d		+3.8 (*c* = 1.0)
		1545		(8.8)	(6.8)		
7d	1725	1680	1645	7.28bs	2.73d		−20.0 (*c* = 0.46)
		1530			(6.8)		

Table 4. The Spectral and Optical Data of **6**

Compd No.	IR, ν/cm^{-1} –NH–	–CONH–	–C=C–	^{1}H NMR, δ α'-H (P_3)	–CH= (*J*/Hz)	α-H (P_1)	$[\alpha]_D^{25}$/° (*c* 1.0, in MeOH)
6c	3358	1737	1665	3.78dd	6.19d	4.25dq	−40.0
		1536		(7.0,7.0)	(10.0)	(7.0,7.5)	
6f	3364	1692	1614	3.99m	6.22d	4.07d	−50.0
		1521			(10.0)	(5.7)	
6g	3310	1665	1640	4.02dd	6.46d	4.46dd	−31.4 (*c* = 1.2)
		1515		(5.7,5.5)	(10.0)	(4.6,5.1)	
6h	3316	1685	1686	4.03dd	6.51d	4.40m	−21.9 (*c* = 1.8)
		1527		(5.5,5.3)	(10.0)		
6l	3334	1686	1635	4.15dq	—	4.42dq	−35.0 (*c* = 0.5)
		1542		(6.6,7.0)		(7.3,7.3)	
6m	3268	1722	1641	3.93dd	—	4.41dq	−63.5
		1524		(6.4,8.1)		(7.3,7.3)	
6n	3394	1720	1650	4.15m	—	4.47dq	−39.1 (*c* = 0.1)
		1539				(7.3,7.3)	
6o	3394	1683	1611	4.11dq	7.31—7.64m	4.38dq	−35.6
		1536		(7.0,6.4)	(+Ph)	(7.3,7.0)	
6p	3344	1724	1642	3.90dd	7.30—7.61m	4.31dq	−45.4 (*c* = 0.9)
		1678		(6.0,7.5)	(+Ph)	(7.0,7.5)	
6q	3334	1722	1647	4.05m	7.19—7.83m	4.30dq	−27.0
		1533			(+Ph)	(7.0,7.0)	

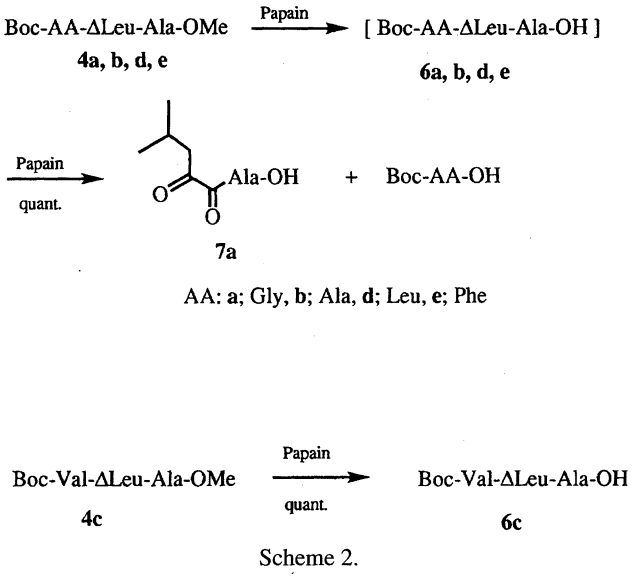
Ala's Me, $J=7.3$ Hz), 2.18 (m, 1H, CH), 2.80d (d, 2H, CH_2, $J=6.8$ Hz), 3.78 (s, 3H, Me), 4.55 (dq, Ala's α-H, $J=7.3$ and 7.5 Hz), 7.42 (br, s, 1H, NH). Found: C, 55.59; H, 7.82; N, 6.65%. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51%.

Enzymatic Coupling of 4 with 5. Typical Procedure: A suspension (4 ml) of Boc–AA–ΔAA–AA–OMe (**4**) (50 mM), an appropriate HCl·H–AA–ΔVal–OMe (**5**) (150 mM) and papain (7.5 g dm^{-3}) in the presence of 2-mercaptoethanol (0.1 ml) in McIlvaine buffer was incubated, with shaking, at pH 6.0 and at 35 °C for 24 h. The intermediate (**5**) was derived from Boc–AA–ΔVal–OMe (150 mM) by treating with EtOAc (1 ml) saturated with dry HCl gas. The deposited colorless crystals were collected by filtration. The filtrate was acidified with 1 M HCl and further diluted with water (30 ml) and then extracted three times with EtOAc (10 ml×3). The combined extracts were washed with brine (30 ml), and then dried over anhydrous $MgSO_4$. Concentration in vacuo gave additional crude crystals. The combined crystals were recrystallized from EtOAc–hexane to give Boc–(*Z*)-$\Delta^{2,5}$-dehydropentapeptide–OMe (**8**—**11**: Boc–AA–ΔAA–AA–AA–ΔVal–OMe) as colorless needles. The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **8**—**11** are summarized in Tables 7 and 8.

Results and Discussion

Enzymatic Ester Hydrolysis of 4. To carry out the ester hydrolysis of **4** using proteolytic enzyme under mild conditions, we used the optimal conditions already obtained in the quite similar hydrolysis of Cbz–ΔAA–AA–OMe.[6] The enzymatic ester hydrolysis of Boc–Leu–ΔLeu–Ala–OMe (**4d**) [200 mM and papain (30 g dm^{-3})] in the presence of 2-mercaptoethanol (0.1 ml) in McIlvaine buffer was performed at pH 8.0 and at 35 °C for 24 h. As a result, although the normal ester hydrolysis giving the hydrolyzate Boc–Leu–ΔLeu–Ala–OH (**6d**) was expected, surprisingly, the unexpected *N*-(4-methyl)pentanedioyl (Mdop)–Ala–OH (**7a**), besides Boc–AA–OH, was obtained quantitatively. On the other hand, in the case using Boc–Val–ΔLeu–Ala–OMe (**4c**) under the similar conditions, only ester hydrolysis occurred to give Boc–Val–ΔLeu–Ala–OH (**6c**) almost quantitatively, as shown in Scheme 2. By comparison with the above two substrates, it is found that there is only one difference between the AA residues at P_3 in **4c** and **4d**.

Furthermore, to study the formation mechanism of **7a**, the hydrolysis of **4d** was examined in regard to the effect of the reaction time, as shown in Fig. 2. That is, the time course of the hydrolysis of **4d** at the optimal pH 8.0 and 35 °C was fully reexamined. As a result, the hydrolyzate Boc–Leu–ΔLeu–Ala–OH (**6d**) was found to increase steeply in the range of the time from 2 to 7 h and then decrease gradually, while in the same time the *N*-Mdop–Ala–OH (**7a**) was also found to increase smoothly to reach quantitative yield for 12 h. On the other hand, however, in the case of **4c**, even though the full examination of the similar hydrolysis was performed, the formation of **7a** was not observed. In addition, to ascertain the formation process of **7a**, Boc–Leu–ΔLeu–D-Ala–OMe (**D-4d**), in place of the L-Ala residue, was sub-



Scheme 2.

Table 7. The Yields and Physical Melting Points of **8**—**11**

Compd No.	Yield %	Mp[a] θ_m/°C	Formula	Found (%) (Calcd) C	H	N
8	60	106—108	$C_{31}H_{53}N_5O_8\cdot 1/2H_2O$	59.01 (58.84	8.66 8.60	10.75 11.06)
9	56	219—220	$C_{34}H_{51}N_5O_8$	62.15 (62.08	7.55 7.81	10.52 10.65)
10	50	222—224	$C_{31}H_{53}N_5O_8$	59.65 (59.69	8.80 8.56	11.52 11.23)
11	51	196—198.5	$C_{30}H_{51}N_5O_8\cdot 1/2H_2O$	58.09 (58.23	8.22 8.47	11.33 11.32)

a) Colorless needles from EtOAc–hexane.

Table 8. The Spectral and Optical Data of **8**—**11**

Compd No.	IR, ν/cm^{-1} –CONH–	–C=C–	^{1}H NMR, δ CONH (*J*/Hz)	–CH=	$[\alpha]_D^{25}$/° (in MeOH)
8	1710	1662	5.14br s, 7.15br s, 7.43br s,	6.03d	−13.0 ($c=1.3$)
	1521		7.57br s, 7.91br s	(10.0)	
9	1690	1658	5.32d (3.5), 7.15—7.33m (+Ph),	5.98d	−36.0 ($c=0.1$)
	1538		7.60d (9.0), 7.72br s, 7.95br s	(10.0)	
10	1690	1650	5.06br s, 7.22—7.31m (CONH × 2),	5.88d	−35.0 ($c=0.2$)
	1524		7.46br s, 7.85br s	(10.0)	
11	1690	1650	3.84—4.44m (+α-H), 5.36d (5.0)	—	−46.4 ($c=1.0$)
	1524		7.19d (4.8), 7.71d (7.7), 8.09d (8.0)		

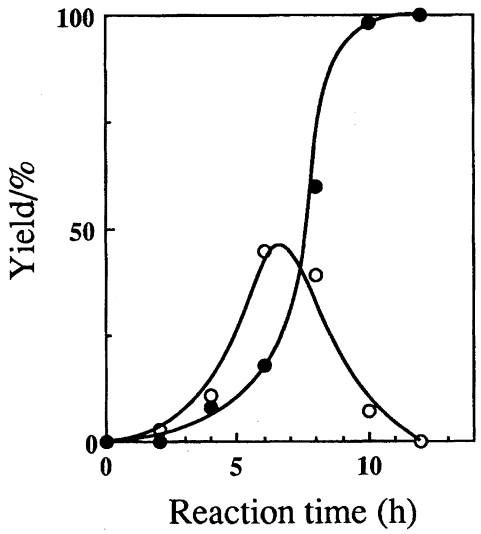
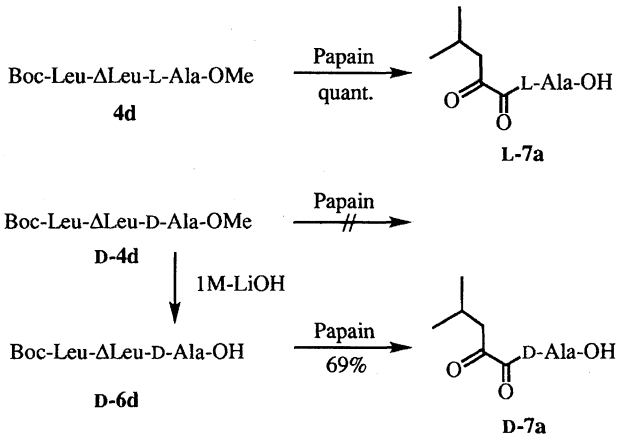


Fig. 2. Effect of reaction time on the yields of **6d** (○) and **7a** (●). The reaction mixture (2.5 ml), containing 200 mM **4d** and 30 g dm^{-3} papain (2.8 units mg^{-1}) in McIlvaine buffer, was shaken at pH 8 at 35°C for various hours.

jected to a similar enzymatic hydrolysis. As a result, according to the expectation, the enzymatic ester hydrolysis did not proceed and the substrate used was completely recovered. However, interestingly, Boc–Leu–ΔLeu–D-Ala–OH (**D-6d**), which was derived by the chemical hydrolysis of the corresponding methyl ester with 1 M LiOH, was hydrolyzed enzymatically at P_2 to give *N*-Mdop–D-Ala–OH (**D-7a**) almost quantitatively, as shown in Scheme 3. To examine whether the above unusual peptide bond cleavages proceed enzymatically or not, the blank tests were carried out. As the results, the bond hydrolysis of **D-6d** as well as **4d** did

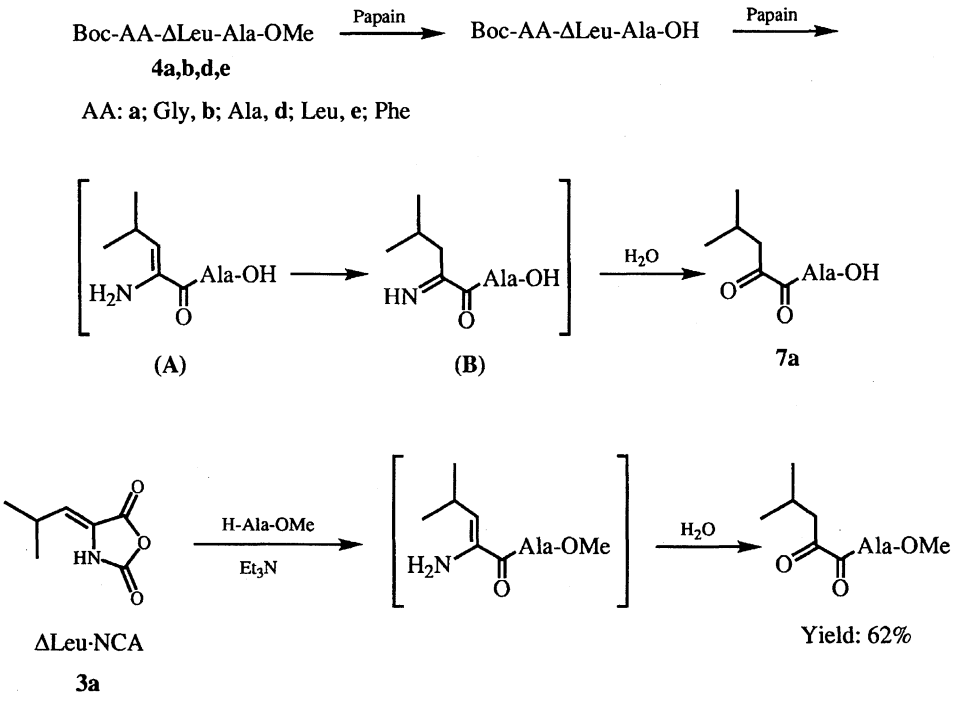


Scheme 3.

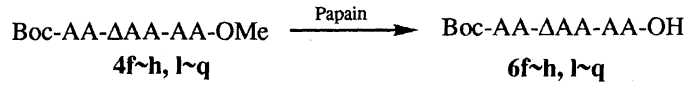
not take place in only McIlvaine buffer without papain, even though the treatment time was longer than 24 h.

From the above results, it is postulated that the formation of **6d** occurred firstly by the hydrolysis of the ester of **4d** and then the selective peptide bond cleavage of **6d** at P_2 took place to form H–ΔLeu–Ala–OH (**A**), which was immediately isomerized to the corresponding 2-imino-4-methylpentanoyl derivative (**B**) and finally the 2-imino group was hydrolyzed to give **7a**. Undoubtedly the latter hydrolysis of **A** to **B** is thought to occur chemically, because the hydrolysis of H–ΔLeu–Ala–OMe,[14] formed by the coupling of **3a** with H–Ala–OMe, gave *N*-Mdop–Ala–OMe, as shown in Scheme 4. Further, the structure of **7a** was clearly determined by the independent preparation by the coupling of H–L-Ala–OMe with 4-methyl-2-oxopentanoic acid by the DCC method and then by the ester hydrolysis using papain.

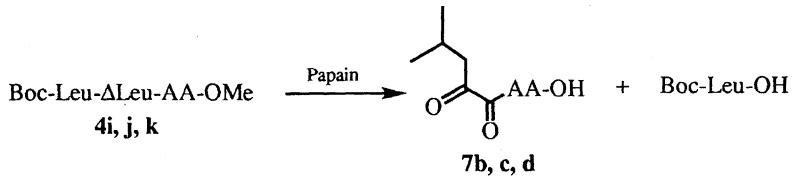
The optimal conditions of the hydrolysis of **4c** and **4d**



Scheme 4.

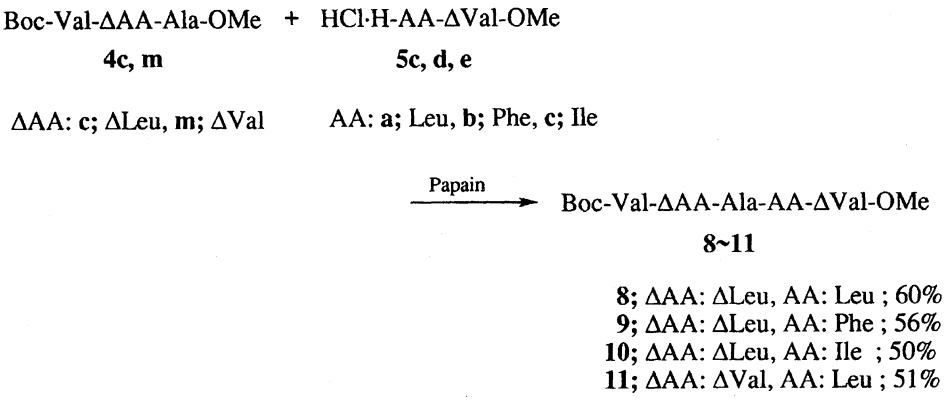


-AA-ΔAA-AA- : **f**; Val-ΔLeu-Gly, **g**; Val-ΔLeu-Val, **h**; Val-ΔLeu-Leu, **l**; Ala-ΔVal-Ala, **m**; Val-ΔVal-Ala, **n**; Leu-ΔVal-Ala, **o**; Ala-ΔPhe-Ala, **p**; Val-ΔPhe-Ala, **q**; Leu-ΔPhe-Ala



AA: **i**; Gly, **j**; Val, **k**; Leu

Scheme 5.



Scheme 6.

[papain (30 g dm^{-3}), substrate (200 mM) in McIlvaine buffer at pH 8.0 and at 35 °C for 24 h] were applied to a similar hydrolysis of all of the substrates **4a—q** (**a**; Gly–ΔLeu–Ala, **b**; Ala–ΔLeu–Ala, **e**; Phe–ΔLeu–Ala, **f**; Val–ΔLeu–Gly, **g**; Val–ΔLeu–Val, **h**; Val–ΔLeu–Leu, **i**; Leu–ΔLeu–Gly, **j**; Leu–ΔLeu–Val, **k**; Leu–ΔLeu–Leu, **l**; Ala–ΔVal–Ala, **m**; Val–ΔVal–Ala, **n**; Leu–ΔVal–Ala, **o**; Ala–ΔPhe–Ala, **p**; Val–ΔPhe–Ala, **q**; Leu–ΔPhe–Ala), as shown in Scheme 5. Consequently, the hydrolyses of seventeen kinds of **4a—q** were fully carried out to give either Boc–AA–ΔAA–AA–OH (**6f—h, l—g**) or *N*-Mdop–AA–OH (**7b, c, d**). As the results, in the two cases of **4** containing Val residue at P_3 and/or ΔVal, ΔPhe residue at P_2, it was found that the ester hydrolysis alone took place predominantly and the further peptide bond cleavage at P_2 did not occur. In these cases, it is presumed that the bulky and rigid residues at P_3 and/or P_2 hindered sterically the enzymatic peptide bond cleavage. Therefore, under the sequential conditions mentioned above, it is expected that the reverse enzymatic coupling of **4** with H–AA–ΔVal–OMe will occur preferentially.

Accordingly, the papain was found to be a sufficiently potent catalyst for the mild ester hydrolysis and peptide bond cleavage of various Δ^2-dehydrotripeptide esters.

The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **6** and **7** are summarized in Tables 3, 4, 5, and 6.

Peptide Bond Formation of 4 with 5. From the above results and the facts obtained so far, the enzymatic peptide bond formation of **4** is also expected. According to the coupling reactions reported previously,[5,6] the following enzymatic coupling reactions were investigated. The coupling of *C*-component Boc–Val–ΔAA–Ala–OMe (**4**) with *N*-component H–AA–ΔVal–OMe (**5**) was tried in order to obtain Boc–$\Delta^{2,5}$-dehydropentapeptide methyl ester (Boc–Val–ΔAA–Ala–AA–ΔVal–OMe (**8—11**)).

By taking advantage of the optimal conditions [papain (7.5 g dm^{-3}), with *C*-component (50 mM) and *N*-component (150 mM) ($N/C = 3.0$) in McIlvaine buffer at pH 6.0 and at 35 °C for 24 h] obtained in the enzymatic coupling of *N*-benzyloxycarbonyl (Cbz)–ΔLeu–Ala–OMe with **5**,[6] the coupling of **4** (ΔAA: **c**; ΔLeu, **m**; ΔVal) with **5** (AA: **c**; Leu, **d**; Phe, **e**; Ile) was performed in McIlvaine buffer using papain (2.8 units/mg), as shown in Scheme 6. As a result, the expected Boc–Val–ΔAA–Ala–AA–ΔVal–OMe (**8**: ΔAA; ΔLeu, AA; Leu. **9**: ΔAA; ΔLeu, AA; Phe. **10**: ΔAA; ΔLeu, AA; Ile. **11**: ΔAA; ΔVal, AA; Leu) was first obtained in ca. 55% yield.

The yields, melting points, and physical constants (IR,

^{1}H NMR, and specific rotation) of **8**—**11** are summarized in Tables 7 and 8.

In conclusion, the present study showed that, from a synthetic standpoints of view, papain was widely useful and served as a tool for the ester hydrolyses of dehydropeptide esters, and their coupling reaction with the other α-amino acid, peptide, and dehydropeptide derivatives under mild conditions. Moreover, the unusual peptide bond cleavage between AA and ΔAA bond could be available to the sequence determination of various unknown natural and synthetic dehydropeptides.

References

1) Part XIX: K. Okumura, A. Ito, H. Saito, Y. Nakamura, and C. Shin, *Bull. Chem. Soc. Jpn.*, **69**, 2309 (1996).

2) C. Shin, N. Takahashi, and Y. Yonezawa, *Chem. Lett.*, **1988**, 2001.

3) C. Shin and N. Takahashi, *Chem. Lett.*, **1989**, 747.

4) C. Shin, N. Takahashi, and M. Seki, *Bull. Chem. Soc. Jpn.*, **64**, 3575 (1991).

5) C. Shin, M. Seki, T. Kakusho, and N. Takahashi, *Bull. Chem. Soc. Jpn.*, **66**, 2048 (1993).

6) C. Shin, T. Kakusho, K. Arai, and M. Seki, *Bull. Chem. Soc. Jpn.*, **68**, 3549 (1995).

7) In this paper, the symbols Δ^1, Δ^2, and $\Delta^{2,5}$ indicate the position number of double bond of DHA residue from the N-terminus in sequence.

8) For examples: J. B. West, W. J. Hennen, J. L. Lalonde, J. A. Bibbs, Z. Zhong, E. F. Meyer, Jr., and C.-H. Wong, *J. Am. Chem. Soc.*, **112**, 5313 (1990); J-M. Fang and C.-H. Wong, *Synlett*, **1994**, 393.

9) For examples: M. Ota, N. Nio, and Y. Ariyoshi, *Biosci. Biotech. Biochem.*, **57**, 808 (1993); M. Schuster, B. Munoz, and C.-H. Wong, *Tetrahedron Lett.*, **34**, 1247 (1993).

10) I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.*, **27**, 157 (1967); **32**, 898 (1968).

11) C. Shin, Y. Yamada, and Y. Yonezawa, *Tetrahedron Lett.*, **24**, 2175 (1983).

12) C. Shin, Y. Yonezawa, and M. Ikeda, *Bull. Chem. Soc. Jpn.*, **59**, 3578 (1986).

13) C. Shin, S. Honda, K. Morooka, and Y. Yonezawa, *Bull. Chem. Soc. Jpn.*, **66**, 1844 (1993).

14) C. Shin, Y. Yonezawa, and T. Yamada, *Chem. Pharm. Bull.*, **32**, 3934 (1984).