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The Reaction of Aspartyl Dipeptide Esters with Ketones

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The methyl and ethyl esters of α-L-aspartyl-L-phenylalanine (I and II), -L-tyrosine (III and IV), -L-(β-cyclohexyl)alanine (V and VI), and -L-[β-(4-hydroxycyclohexyl)]alanine (VII and VIII) have been found to react with ketones (acetone, methyl ethyl ketone, and cyclohexanone), in the absence of any catalysts, to give the corresponding 4-imidazolidinone derivatives (XVII—XXX). The NMR spectra of the imidazolidinones with an aromatic side chain (XVII—XXVIII) have indicated that these compounds exist in the folded conformation (in which the aromatic ring faces the imidazolidinone ring) in the solution. The imidazolidinones (XVII—XXX) were hydrolyzed with hot water to give the peptide esters (I—VIII), all in their original optical purity. On the other hand, the β-isomers of the peptide esters (IX—XVI) did not react with these ketones under the same conditions. These characteristics were used successfully in the separation of the two structural isomers of these peptide esters.

The methyl and ethyl esters of α-L-aspartyl-L-phenylalanine (I and II), -L-tyrosine (III and IV), -L-(β-cyclohexyl)alanine (V and VI), and -L-[β-(4-hydroxycyclohexyl)]alanine (VII and VIII) all have a sweet taste very similar to that of sucrose, and they have recently become well known as sweetening agents.[1,2]

In the course of studies of these sweet peptides, it was found that the dipeptide esters (I—IV) readily reacted with ketones, such as acetone, methyl ethyl ketone, and cyclohexanone, in the absence of any catalysts, to give crystalline 4-imidazolidinone derivatives (XVII—XXVIII) (Fig. 1 and Table 2). However, most of the reactions of the hexahydroderivatives (V—VIII), obtained by the hydrogenation of the aromatic rings of the corresponding dipeptide esters (I—IV) with hydrogen and platinum catalyst, with these ketones gave oily products. Among them, only the reactions of V and VI with acetone gave analytically pure crystalline products (XXIX and XXX). Similar reactions have been observed in the cases of carbobenzoxyamino acid amides,[3] ampicillin,[4] and oxytocin.[5]

The structures of the reaction products (XVII—XXX) were determined on the basis of their analytical and spectral data and their chemical behavior. The results of the elemental analyses of the reaction products were in agreement with the values calculated for the imidazolidinones (XVII—XXX) (Table 2). The IR spectra of the imidazolidinones containing the phenylalanyl or the β-cyclohexylalanyl residue (XVII—XXII, XXIX, and XXX) showed two carbonyl absorptions at 1740—1750 cm^{-1} (ester) and at 1700—1703 cm^{-1} (a composite band of the amide and carboxyl groups), whereas the imidazolidinones containing the tyrosyl residue (XXIII—XXVIII) showed two carbonyl absorptions at 1720—1725 cm^{-1} (a composite band of the ester and carboxyl groups) and 1695 cm^{-1} (amide). All of these imidazolidinones (XVII—XXX) lacked amide-II absorptions of the starting materials in the region of 1500—1600 cm^{-1}.

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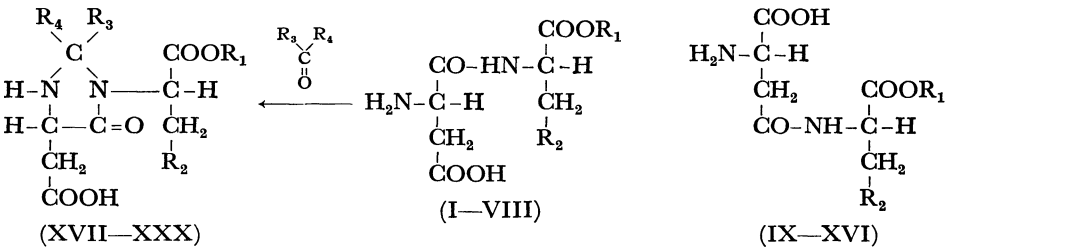
2) J. M. Schlatter, German Pat., 1936159 (1970).
3) U. Zehavi and D. Ben-Ishai, *J. Org. Chem.*, **26**, 1097 (1961).
4) G. A. Hardcastle, Jr., D. A. Johnson, and C. A. Panetta, *ibid.*, **31**, 897 (1966).
5) V. J. Hruby, D. Yamashiro, and V. du Vigneaud, *J. Amer. Chem. Soc.*, **90**, 7106 (1968).

Table 1. Dipeptide esters used in this experiment

Compound	Mp °C	$[\alpha]_D$ (water)	Found %			Calcd %				Lit	
			C	H	N	Formula	C	H	N	Mp °C	$[\alpha]_D$ (water)
I	235—236	+32.0[a]	55.47	6.21	9.35	$C_{14}H_{18}O_5N_2 \cdot 1/2H_2O$	55.44	6.31	9.24	246—247[1),b)]	0[1),b)]
II	236—237	+25.0[a]	56.73	6.89	8.62	$C_{15}H_{20}O_5N_2 \cdot 1/2H_2O$	56.77	6.67	8.83	244—246[1)]	−6[1),c)]
III	173—174	+5.5	54.40	6.14	8.91	$C_{14}H_{18}O_6N_2$	54.19	5.85	9.03	180—185[1)]	+4[1)]
IV	174—175	+5.0	55.93	6.43	8.47	$C_{15}H_{20}O_6N_2$	55.55	6.22	8.64	189—190[1)]	+20[1),d)]
V	119—120	−10.5	51.49	8.58	8.41	$C_{14}H_{24}O_5N_2 \cdot 3/2H_2O$	51.36	8.31	8.56	130—137[2)]	−13.5[2)]
VI	117—118	−17.0	55.54	8.64	8.49	$C_{15}H_{26}O_5N_2 \cdot 1/2H_2O$	55.71	8.42	8.66		
VII	149—150	−11.5	53.48	7.84	8.69	$C_{14}H_{24}O_6N_2$	53.15	7.65	8.86	101—135[2)]	−12.5[2)]
VIII	102—105	−15.5	54.77	7.96	8.42	$C_{15}H_{26}O_6N_2$	54.53	7.93	8.48		
IX	198—199	+40.5[a]	55.37	6.43	9.31	$C_{14}H_{18}O_5N_2 \cdot 1/2H_2O$	55.44	6.31	9.24	196—197[1)]	+4[1)]
X	182—183	+1.0	55.12	6.99	8.74	$C_{15}H_{20}O_5N_2 \cdot H_2O$	55.20	6.80	8.58		
XI	197—198	+14.5	54.37	5.93	9.13	$C_{14}H_{18}O_6N_2$	54.19	5.85	9.03	202—204[1)]	+13[1)]
XII	200—205	+10.5	52.62	6.34	7.90	$C_{15}H_{20}O_6N_2 \cdot H_2O$	52.62	6.48	8.18		
XIII	164—165	−30.0	55.54	8.61	8.08	$C_{14}H_{24}O_5N_2 \cdot C_2H_5OH$	55.47	8.78	8.09		
XIV	188—189	−26.5	55.49	8.17	8.74	$C_{15}H_{26}O_5N_2 \cdot 1/2H_2O$	55.71	8.42	8.66		
XV	75— 85	−25.5	53.60	7.59	8.89	$C_{14}H_{24}O_6N_2$	53.15	7.65	8.86		
XVI	127—130	−28.5	54.64	8.11	8.24	$C_{15}H_{26}O_6N_2 \cdot 1/2C_2H_5OH$	54.37	8.27	7.93		

a) Measured in acetic acid. b) Ref. 10, mp 246—247°, $[\alpha]_D$ −2.3 (NHCl). c) Methanol. d) 75% Acetic acid.



(XVII—XXX) (I—VIII) (IX—XVI)

Dipeptide	R_1	R_2	Dipeptide	R_1	R_2
I	CH_3	C_6H_5	IX	CH_3	C_6H_5
II	C_2H_5	C_6H_5	X	C_2H_5	C_6H_5
III	CH_3	C_6H_5O	XI	CH_3	C_6H_5O
IV	C_2H_5	C_6H_5O	XII	C_2H_5	C_6H_5O
V	CH_3	C_6H_{11}	XIII	CH_3	C_6H_{11}
VI	C_2H_5	C_6H_{11}	XIV	C_2H_5	C_6H_{11}
VII	CH_3	$C_6H_{11}O$	XV	CH_3	$C_6H_{11}O$
VIII	C_2H_5	$C_6H_{11}O$	XVI	C_2H_5	$C_6H_{11}O$

Imidazolidinone	R_1	R_2	R_3	R_4
XVII	CH_3	C_6H_5	CH_3	CH_3
XVIII	CH_3	C_6H_5	CH_3	C_2H_5
XIX	CH_3	C_6H_5	$(CH_2)_5$	
XX	C_2H_5	C_6H_5	CH_3	CH_3
XXI	C_2H_5	C_6H_5	CH_3	C_2H_5
XXII	C_2H_5	C_6H_5	$(CH_2)_5$	
XXIII	CH_3	C_6H_5O	CH_3	CH_3
XXIV	CH_3	C_6H_5O	CH_3	C_2H_5
XXV	CH_3	C_6H_5O	$(CH_2)_5$	
XXVI	C_2H_5	C_6H_5O	CH_3	CH_3
XXVII	C_2H_5	C_6H_5O	CH_3	C_2H_5
XXVIII	C_2H_5	C_6H_5O	$(CH_2)_5$	
XXIX	CH_3	C_6H_{11}	CH_3	CH_3
XXX	C_2H_5	C_6H_{11}	CH_3	CH_3

Fig. 1. Dipeptides and 4-imidazolidinones

When the NMR spectra of the compounds with an aromatic side chain (XVII—XXVIII) were measured in acetone-d_6 or dimethyl sulfoxide-d_6, the protons of one of the two alkyl groups on the C-2 carbon were found to be shifted to a higher field (appearing at δ 0.4—0.6 ppm) by the magnetic shielding effect of the aromatic ring; for example, the H_a and H_b protons of the 2,2-dimethyl group of XVII appeared at δ 0.57 ppm (3H, singlet) and δ 1.33 ppm (3H, singlet) respectively in acetone-d_6, whereas the corresponding six protons of the hexahydroderivative (XXIX) were found at δ 1.45 ppm as a singlet. The two protons on the β-carbon atom of the aspartyl residue of XVII were almost unaffected (Fig. 2). Therefore, this shift suggests that

Table 2. 4-Imidazolidinones

Compound	Yield %	Mp °C	Found %			Calcd %			
			C	H	N	Formula	C	H	N
XVII	61	145[a)]	60.91	6.80	8.24	$C_{17}H_{22}O_5N_2$	61.06	6.63	8.38
XVIII	67	140[a)]	62.17	7.18	8.05	$C_{18}H_{24}O_5N_2$	62.05	6.94	8.04
XIX	81	207—208[b)]	64.08	7.18	7.50	$C_{20}H_{26}O_5N_2$	64.15	7.00	7.48
XX	34	142[a,b)]	62.06	6.98	8.13	$C_{18}H_{24}O_5N_2$	62.05	6.94	8.04
XXI	22	138—139[a)]	62.88	7.33	7.78	$C_{19}H_{26}O_5N_2$	62.96	7.23	7.73
XXII	54	171—172[b)]	64.95	7.49	7.25	$C_{21}H_{28}O_5N_2$	64.93	7.27	7.21
XXIII	95	139.5—140[b)]	58.30	6.64	7.70	$C_{17}H_{22}O_6N_2$	58.27	6.33	8.00
XXIV	54	169[b)]	59.45	6.67	7.72	$C_{18}H_{24}O_6N_2$	59.33	6.64	7.69
XXV	84	191.5[b)]	61.23	6.95	7.10	$C_{20}H_{26}O_6N_2$	61.52	6.71	7.18
XXVI	67	151[b)]	59.45	6.78	7.53	$C_{18}H_{24}O_6N_2$	59.33	6.64	7.69
XXVII	41	151.5—152.5[b)]	60.28	7.17	7.46	$C_{19}H_{26}O_6N_2$	60.30	6.93	7.40
XXVIII	35	178[b)]	62.39	7.13	6.83	$C_{21}H_{28}O_6N_2$	62.36	6.98	6.93
XXIX	54	114—115	59.96	8.47	8.34	$C_{17}H_{28}O_5N_2$	59.98	8.29	8.23
XXX	45	125—126	60.91	8.35	7.62	$C_{18}H_{30}O_5N_2$	60.99	8.53	7.90

a) Raised rapidly. b) Decomposition.

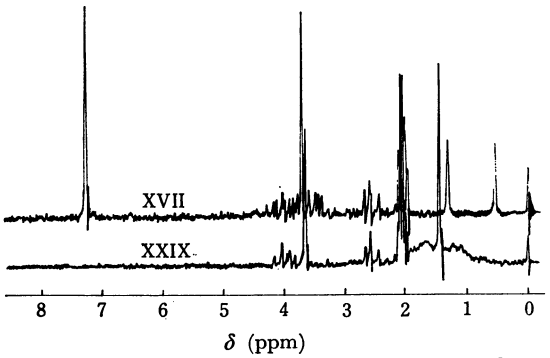


Fig. 2. NMR spectra of XVII and XXIX (in CD_3COCD_3).

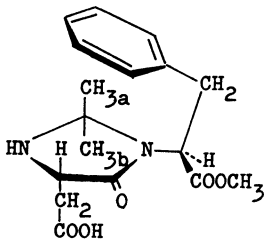


Fig. 3. Preferred conformation of XVII

compound XVII takes the folded conformation, in which the aromatic ring faces the imidazolidinone ring, as is shown in Fig. 3.

In boiling water, all the imidazolidinones except for the spiroderivatives were completely hydrolyzed within twenty minutes to give the corresponding peptide esters in their original optical purity. The spiroderivatives (XIX, XXII, XXV, and XXVIII) were much more stable to hydrolysis. Under the same conditions, they were only partly hydrolyzed to the corresponding peptide esters. Some two hours were required for their complete hydrolysis.

The reactions of the peptide esters (I—VIII) with aldehydes, such as acetaldehyde, propionaldehyde, or isobutylaldehyde, failed to give crystalline products.

The corresponding β-isomers of the peptide esters (IX—XVI), which have a bitter taste, did not react with these ketones under the same conditions. These characteristics were used successfully in the separation of the two structural isomers of these sweet peptide esters. When these peptides were conveniently prepared by the condensation of such *N*-protected aspartic anhydrides as carbobenzoxy-L-aspartic anhydride with the appropriate amino acid esters, followed by deprotection, they were always mixtures of α- and β-aspartyl peptides. Several methods of separating α- and β-aspartyl peptides have been reported, including techniques of fractional extraction,[6] column chromatography,[7] and selective precipitation.[8]

When a mixture of the α- and β-aspartyl peptide esters (I and IX, II and X, III and XI, IV and XII, V and XIII, or VIII and XVI) was treated in acetone, the α-isomer changed to the corresponding soluble imidazolidinone, whereas the unchanged β-isomer remained insoluble. The β-isomer was filtered and recrystallized from water to give the pure β-isomer. The filtrate from the β-isomer was concentrated to dryness to give the imidazolidinone, which was then hydrolyzed with hot water to afford the pure α-isomer. The results are summarized in Table 3.

Experimental

All the melting points are uncorrected. The IR spectra were recorded in Nujol mull with a Jasco IR-S spectrometer. The NMR spectra were obtained with a Varian T-60 spectrometer at 60 MHz, and the chemical shifts are given from tetramethylsilane as the internal reference. The structural isomers were determined by a method described previously.[8]

Preparation of the Starting Materials. The α-L-aspartyl-L-phenylalanine methyl and ethyl esters (I and II), and the α-L-aspartyl-L-tyrosine methyl and ethyl esters (III and IV) were prepared by the condensation of β-benzyl *N*-carbobenzoxy-L-aspartate[9] and the appropriate amino acid esters

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8) Y. Ariyoshi and N. Sato, This Bulletin, **45**, 942 (1972).
9) L. Benoiton, *Can. J. Chem.*, **40**, 570 (1962).

Table 3. Separation of the α- and β-aspartyl peptide esters

Run No.	Mixture of α- and β-isomers				Reaction conditions			Recovery of pure isomers			
	α-isomer,	g	β-isomer,	g	acetone ml	temp.	time hr	α-isomer,	g (%)	β-isomer,	g (%)
1	I	4	IX	4	200	r.t.	15	I	3.3 (83)	IX	3.3 (83)
2	II	1	X	1	100	reflux	1	II	0.8 (80)	X	0.6 (60)
3	III	1	XI	1	100	reflux	2.5	III	0.7 (70)	XI	0.7 (70)
4	IV	1	XII	1	100	reflux	2.5	IV	0.7 (70)	XII	0.6 (60)
5	V	0.5	XIII	0.5	25	r.t.	0.5	V	0.2 (40)	XIII	0.4 (80)
6	VIII	0.5	XVI	0.5	25	r.t.	0.5	VIII	0.2 (40)	XVI	0.4 (80)

with ethyl chloroformate, followed by deprotection. A typical run (I in Table 1) was as follows: To a chilled solution of β-benzyl *N*-carbobenzoxy-L-aspartate (17.9 g) and triethylamine (5.1 g) in chloroform (120 m*l*) was added ethyl chloroformate (5.5 g) at −5——10°C with stirring. After the solution had been stirred for 20 min at this temperature, a chilled mixture of methyl L-phenylalaninate hydrochloride (11.9 g) and triethylamine (6.5 g) in chloroform (150 m*l*) was stirred into the mixed anhydride solution at −5——10°C. Stirring was continued for 3 hr at room temperature. The reaction mixture was washed with N hydrochloric acid and then water, and dried over sodium sulfate. The solvent was removed by distillation *in vacuo*, and the residue was recrystallized from ethyl acetate-petroleum ether to give β-benzyl *N*-carbobenzoxy-L-aspartyl-L-phenylalanine methyl ester as needles; yield, 21 g (81%); mp 114.5—115.5°C. (Found: C, 67.16; H, 6.13; N, 5.43%). lit, 116—117°C,[10] 118.5—119.5°C.[1] A solution of the dipeptide ester (8.3 g) in 75% aqueous acetic acid (150 m*l*) was hydrogenated in the presence of 5% palladium on charcoal (1.0 g) for 6 hr. The filtrate from the catalyst was then concentrated *in vacuo*, and the residual crystals were recrystallized from water to give I as needles; yield, 4.0 g (83%). The data are given in Table 1.

The β-L-aspartyl-L-phenylalanine methyl and ethyl esters (IX and X), and the β-L-aspartyl-L-tyrosine methyl and ethyl esters (XI and XII) were prepared from α-benzyl *N*-carbobenzoxy-L-aspartate[11] and the appropriate amino acid esters according to the method used in the preparation of the α-isomers. A typical run (IX in Table 1) was as follows: α-Benzyl *N*-carbobenzoxy-L-aspartate (7.2 g) was condensed with methyl L-phenylalaninate hydrochloride (5.2 g) by the method described in the preparation of the α-isomers; this gave 7.8 g (75%) of α-benzyl *N*-carbobenzoxy-L-aspartyl-L-phenylalanine methyl ester; mp 121—122°C. lit,[1] mp 131—133°C. (Found: C, 67.37; H, 6.05; N, 5.45%). The hydrogenolysis of 10 g of the dipeptide ester gave 3.8 g of IX. All of the compounds (IX—XII) were hygroscopic. The data are given in Table 1.

The α-L-aspartyl-L-(β-cyclohexyl)alanine methyl and ethyl esters (V and VI), the α-L-aspartyl-L-[β-(4-hydroxycyclohexyl)]alanine methyl and ethyl esters (VII and VIII), the β-L-aspartyl-L-(β-cyclohexyl)alanine methyl and ethyl esters (XIII and XIV), and the β-L-aspartyl-L-[β-(4-hydroxycyclohexyl)]alanine methyl and ethyl esters (XV and XVI) were obtained by the hydrogenation of the aromatic ring of the corresponding dipeptides (I—IV and IX—XII). A typical run (V in Table 1) was as follows: A solution of I (5.0 g) in 0.1M aqueous acetic acid (250 m*l*) was hydrogenated in the presence of platinum oxide (1.0 g) at atmospheric pressure and room temperature. The filtrate from the catalyst was then concentrated to dryness *in vacuo*. The residue was triturated with ether to give V as a crystalline powder, which was then recrystallized from water to give platelets; 4.8 g (94%).

VIII, XIV, XV, and XVI were hygroscopic. The data are given in Table 1.

4-Imidazolidinones (XVII—XXX). The imidazolidinones (XVII—XXX) were prepared by the condensation of the dipeptides (I—VIII) with acetone, methyl ethyl ketone, or cyclohexanone at 25—100°C for 0.5—12 hr. A typical run (XVII in Table 2) was as follows: A suspension of I (6.0 g) in acetone (100 m*l*) was heated under reflux for 30 min. The insoluble crystals (1.5 g) were then filtered off. The crystals were identified with I by studying the IR spectrum. After the filtrate had been kept in a refrigerator overnight, the crystals thus formed were collected by filtration; yield, 3.0 g. The second crop (1.0 g) was recovered from the mother liquor; total yield, 4.0 g (61%); mp 145°C (raised rapidly); $[\alpha]_D^{25}$ −157.5° (*c* 1, acetone); IR: 3310 (NH), 1750 (ester), and 1703 (amide-I and carboxyl group) cm^{-1}; NMR (CD_3COCD_3): δ 0.57 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 2.57 (m, 2H, $-CH_2COO$), 3.50 (m, 2H, $-CH_2C_6H_5$), 3.73 (s, 3H, $-COOCH_3$), 3.50—4.20 (m, 2H, 2 $-CH\langle$), 4.37 (broad s, NH), and 7.30 (s, 5H, $-C_6H_5$) ppm. For the NMR spectrum, see also Fig. 2. For anal. and mp, see Table 2.

Separation of the α- and β-Isomers with Acetone. A typical run (No. 1 in Table 3) was as follows: A suspension mixture of I (4 g) and IX (4 g) in acetone (200 m*l*) was stirred for 15 hr at room temperature. An insoluble, amorphous material was then collected by filtration. The compound was identified as anhydrous IX by studying the IR spectrum, which failed to absorb the water of crystallization of the starting material. The compound was recrystallized from water (40 m*l*) to give 3.3 g (83%) of IX as needles, which were found by paper electrophoresis to be free from the α-isomer; mp 198—199°C (decomp.); $[\alpha]_D^{22}$ +40.5° (*c* 1, acetic acid).

On the other hand, the filtrate from the β-isomer was concentrated to dryness *in vacuo*. The residual crystals were dissolved in hot water, concentrated to dryness *in vacuo*, and then recrystallized from water (40 m*l*) to give 3.3 g (83%) of I as needles; mp 235—236°C (decomp.); $[\alpha]_D^{25}$ +32.0° (*c* 1, acetic acid). These results showed that no racemization occurred during the reaction. The crystals were found by paper electrophoresis to be free from the β-isomer. The results are summarized in Table 3.

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