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The Use of Esters of Simple Ketoximes in Peptide Synthesis

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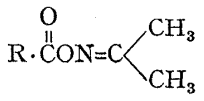
A number of esters of carbobenzoxyamino acids with acetoxime, cyclopentanonoxime and cyclohexanonoxime have been synthesized by direct condensation using dicyclohexylcarbodiimide. These esters are stable and crystalline solids (including carbobenzoxy-serine, -threonine and -nitroarginine) and react readily with amino acid esters or peptide esters in the presence of acid catalyst such as acetic acid, formic acid or phenylacetic acid.

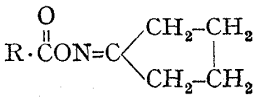
Because of the easy availability and the water solubility of the simple ketoximes, these active esters should be useful for the peptide synthesis.

In recent years, the active esters such as *p*-nitrophenyl esters,[2] 2,4,5-trichlorophenyl esters,[3] N-hydroxysuccinimide esters[4] and N-hydroxypiperidine esters[5] of amino acids or peptides have been frequently used in the field of peptide synthesis. The latter two esters are particularly interesting, because the coproduct, N-hydroxysuccinimide or N-hydroxypiperidine is readily removed from the product by washing with water.

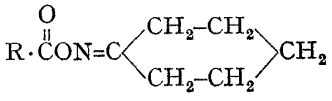
In the present study, we found that esters of simple ketoximes of acylamino acids, *e.g.* esters of acetoxime (I), cyclopentanonoxime (II) or cyclohexanonoxime (III), reacted smoothly with an amino component in the presence of acetic acid to give acylpeptide derivatives with high purity in good yields.



I



II



III

Losse, *et al.*[6] originally prepared O-(carbobenzoxy-glycyl)-acetoxime and -cyclohexanonoxime in 1964, and reported that these esters were slowly condensed with benzylamine and glycine ethyl ester, and the desired peptides were given in 61% yield.

First, I, II and III of various carbobenzoxyamino acids were prepared from the carbobenzoxyamino acids and the ketoximes by the dicyclohexylcarbodiimide method. As can be seen in Table I, most of the esters are stable and colorless crystals.

The reactivity of O-(carbobenzoxy-L-alanyl)-acetoxime and -cyclohexanonoxime with glycine ethyl ester in chloroform were checked, but the reactions were too slow to use in the peptide synthesis.[7] Then, following an observation made by Young, *et al.*,[5] the reactions

1) Location: *Juso-nishinocho, Higashiyodogawa-ku, Osaka.*
2) M. Bodanszky, *Nature*, **175**, 685 (1955).
3) J. Pless and R.A. Boissonnas, *Helv. Chim. Acta*, **66**, 1609 (1963).
4) G.W. Anderson, J.E. Zimmerman and F.M. Callahan, *J. Am. Chem. Soc.*, **85**, 3039 (1963); *ibid.*, **86**, 1839 (1964).
5) S.M. Beaumont, B.O. Hanford, J.H. Jones and G.T. Young, *Chem. Commun.*, **1963**, 53; B.O. Hanford, J.H. Jones, G.T. Yonug and (in part) T.F.N. Johnson, *J. Chem. Soc.*, **1965**, 6814; J.H. Jones and G.T. Young, *J. Chem. Soc.*, (C), **1968**, 53.
6) G. Losse, A. Barth and K. Schatz, *Ann. Chem.*, **677**, 185 (1964).
7) 3 or 4 days were required for completion of the coupling reactions at room temperature.

Table I. Preparation of O-(Carbobenzoxy amino acyl)-ketoxime

O-(Z-Amino acyl) -ketoxime	Yield (%)	mp (°C)	$[\alpha]_D^{24}$ (c=1.0, in $CHCl_3$)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
(I) Acetoxime									
L-Alanine	89	60.5—61.5	+17.0	60.42	6.25	10.07	60.51	6.54	10.00
L-Nitroarginine	87	109.0—112.0	−28.0	49.99	5.92	20.58	49.86	5.93	20.51
S-Benzyl-L-cysteine	99	99—100	− 9.0	62.99	6.04	7.00	62.93	6.09	7.06
Glycine	98	109—110[a]		59.08	6.10	10.60	59.25	6.21	10.59
L-Phenylalanine	86	62—63	+14.4	67.78	6.26	7.91	67.82	6.20	7.96
D-Phenylglycine	98	63.5—65.0	−54.0	67.04	5.92	8.23	67.19	6.16	7.83
L-Phenylalanylglycine	97	126—127	− 4.5	64.22	6.12	10.21	64.44	6.19	10.21
L-Serine	84	83—85	− 2.3	57.13	6.17	9.52	57.06	6.15	9.51
L-Threonine	92	95.5—97.5	−12.8	68.43	6.54	9.09	58.68	6.60	9.14
(II) Cyclopentanonoxime									
L-Alanine	78	106.5—107.5	+19.0	63.14	6.62	9.21	63.36	6.59	9.23
S-Benzyl-L-cysteine	87	123—124	−11.9	64.17	6.15	6.57	64.42	6.08	6.59
Glycine	97	120.5—121.5		62.05	6.25	9.65	62.36	6.46	9.60
-Z-L-Lysine	98	83.0—84.5	+ 9.0	65.44	6.71	8.48	65.33	6.87	8.46
L-Phenylalanine	98	73—74	+ 8.7	69.45	6.36	7.36	69.63	6.52	7.33
L-Valine	85	89—90	+30.6	65.04	7.28	8.43	65.20	7.29	8.37
(III) Cyclohexanonoxime									
L-Alanine	94	65—66	+21.0	64.13	6.97	8.80	64.31	7.09	8.76
L-Nitroarginine·MeOH	95	86—89	−23.0	52.49	6.71	17.49	52.28	6.42	17.79
S-Benzyl-L-cysteine	91	67—68	− 6.6	65.44	6.41	6.36	65.33	6.45	6.41
Glycine	71	81—82[b]		63.14	6.62	9.21	62.92	6.54	9.35
L-Phenylalanine	76	54—55	+ 8.7	70.03	6.64	7.10	69.94	6.84	7.11
D-Phenylglycine	95	100—101	−51.2	69.45	6.36	7.36	69.48	6.64	7.30
L-Serine	95	88—90	+ 4.3	61.06	6.64	8.38	60.86	6.70	8.40
L-Threonine	94	91.5—92.5	− 3.5	62.09	6.94	8.04	62.00	7.02	7.90
L-Valine	68	62—63	+33.0	65.87	7.57	8.09	65.68	7.78	8.14

a) G. Losse, *et al.*[6] give mp 110—112°. *b*) G. Losse, *et al.*[6] give mp 80.8—81.5°.
z: carbobenzoxy-

were carried out in the presence of the acetic acid catalyst.[8] Under this coupling condition, the reactions of ketoxime esters were also remarkably accelerated (see Exp. 1—4 in Table II). As are summarized in Table II, we have prepared some dozen of peptide derivatives by this method. The yields of the reaction products were always excellent and their purities were also quite satisfactory.

The most significant advantage of the simple ketoxime method is that the method can be applied for N-carbobenzoxy-nitroarginine, -serine and -threonine. Although the pentachlorophenyl ester and 2,4-dinitrophenyl ester of carbobenzoxy-nitro-L-arginine were reported by Kovacs, *et al.*[9] and Bodanszky, *et al.*[10] respectively, the pentachlorophenyl ester gave poor yields of the desired peptides and the 2,4-dinitrophenyl ester was a noncrystallizable solid, and one additional disadvantage of both ester was the insolubility of by-product (pentachlorophenol and 2,4-dinitrophenol) in water. In contrast with the above esters, I and III of carbobenzoxy-nitro-L-arginine could be prepared easily as crystalline products by the usual procedure in good yields (85—95%), and reacted smoothly with an equivalent of amino acid

8) The reactions were also accelerated by formic acid or phenylacetic acid. The reactions were somewhat inhibited by 1 equivalent of triethylamine.
9) J. Kovacs and M.Q. Ceprini, *Chem. Ind.* (London), **1965**, 2100.
10) M. Bodanszky and M.A. Ondetti, *Chem. Ind.* (London), **1966**, 26.

Table II. The Synthesis of Peptide using Esters of Simple Ketoximes

No.	Active ester	Amino component	Solvent (equivalent of acetic acid)	Reaction time (hr)	Yield (crude yield)	mp (°C) Found	mp (°C) Lit.	$[\alpha]_D$ (temp. conc. solvent) Found	$[\alpha]_D$ (temp. conc. solvent) Lit.
1.	Z–Ala–O– (I)	H–Gly–OEt	$CHCl_3$ (1)	3	87 (90)	98—99	99—99.5[12]	−21.7	−21.3[13] (25, 1, EtOH)
2.	Z–Ala–O– (I)	H–Gly–OEt	$CHCl_3$ (0.2)	3	87 (90)	98—99	99—99.5	−21.6	−21.3 (25, 1, EtOH)
3.	Z–Ala–O– (I)	H–Gly–OEt	$CHCl_3$ (–)	72	85 (89)	98—99	99—99.5	−21.6	−21.3 (25, 1, EtOH)
4.	Z–Ala–O– (III)	H–Gly–OEt	$CHCl_3$ (1)	3	89 (91)	98—99	99—99.5	−22.0	−21.3 (25, 1, EtOH)
5.	Z–Ala–O– (I)	H–Phe–OEt	$CHCl_3$–DMF (1)	7	85 (91)	99—99.5	97—98[14]	−18.0 (25, 1, EtOH)	
6.	Z–Ala–O– (II)	H–Phe–OEt	$CHCl_3$–DMF (1)	12	82 (86)	99—99.5	87—98	−18.0 (25, 1, EtOH)	
7.	Z–NO_2–Arg–O– (III)	H–Gly–OBz	$CHCl_3$ (1.5)[a]	24	85 (92)	145—147	153.5—154.5[15]	−14.7 (23, 1, MeOH)	−13.9 (25, 1, MeOH)
8.	Z–NO_2–Arg–O (I)	H–Phe–OEt	dioxane (1.5)	24	98 (100)	142—143.5	141—143[15]	− 6.0 (22, 1, DMF)	
9.	Z–NO_2–Arg–O– (I)	H–Pro–OBz	dioxane (1.5)	24	97 (100)	147—148.5	147—148.5[9]	−41.3 (22, 1, DMF)	−42.0[9] (30, 1, DMF)
10.	Z–Cys(Bz)–O– (I)	H–Gly–OEt	$CHCl_3$ (1)	5	95 (98)	97—98.5	98—99[16]	−28.3	−26.8[16] (24, 6, AcOH)
11.	Z–Cys(Bz)–O– (III)	H–Gly–OEt	$CHCl_3$ (0.2)	10	93 (94)	97—98.5	98—99	−27.7 (24, 1, AcOH)	
12.	Z–Gly–O– (I)	H–Gly–OEt	DMF (1)	4	87 (95)	82—83	82.5—83[17]		
13.	Z–Gly–O– (III)	H–Gly–OEt	DMF (0.2)	10	85 (88)	82—83	82.5—83		
14.	Z–Gly–O– (II)	H–Phe–OBz	CH_2Cl_2 (0.2)	10	99 (100)	74	74[18]	− 4.5 (25, 1, EtOH)	
15.	Z–Gly–O– (II)	H–Ser–OMe	DMF (1)	10	73 (82)	94—96	96[19]	− 4.0 (24, 1, EtOH)	
16.	Z–Gly–O– (III)	H–Ala–Gly–OEt	DMF (1)	12	84 (90)	144—145	145[20]	−31.3 (25, 1, EtOH)	
17.	Z–Phe–O– (I)	H–Gly–OEt	$CHCl_3$–DMF (1)	12	90 (92)	110.5—111.5	110—111[21]	−16.0	−16.9[21] (25, 6, EtOH)
18.	Z–Phe–O– (III)	H–Phe–OBz	$CHCl_3$ (0.2)	12	88 (90)	156—157	149—150[22]	+ 9.1	+ 8.4[22] (25, 2, $CHCl_3$)
19.	Z–Ser–O– (III)	H–Gly–OMe	$CHCl_3$ (1)	16	84 (92)	102—103	105—106[23]	− 8.7 (23, 1, MeOH)	
20.	Z–Ser–O– (I)	H–Tyr–OMe	Dioxane (1)	12	93 (100)	113—114	112—113[19]	+12.0	+13.5[24] (23, 2, DMF)
21.	Z–Thr–O– (III)	H–Gly–OMe	$CHCl_3$ (1)	16	86 (90)	107—109.5	105—107[23]	−15.0	−13.9[23] (23, 1, MeOH)
22.	Z–Val–O– (II)	H–Gly–OEt	$CHCl_3$–Dioxane (1)	12	83 (85)	165—166	166[25]	−26.5	−25.3[5] (24, 1, EtOH)

a) Formic acid was used.
Z, carbobenzoxy Bz, benzyl Et, ethyl Me, methyl DMF, dimethylformamide
The amino acids (except glycine) in this Table are of the L-configuration.

esters in the presence of acetic acid (1.5 eq.) and gave the desired nitroarginyl-peptide derivatives in excellent yields (Exp. 7—9 in Table II).

I and II of carbobenzoxy-L-serine and -L-threonine were also obtained in good yields without protection of the hydroxyl group in the side-chain, and these active esters could be successfully used for the preparation of carbobenzoxy-L-seryl- and -L-threonyl peptides (Exp. 19—21 in Table II).

Because of the water solubility[11] and the easy availability of the simple ketoximes, these esters appear to be quite useful in the peptide synthesis.

Experimental[26]

O-(N-Carbobenzoxy-L-nitroarginyl)acetoxime——N-Carbobenzoxy-L-nitroarginine (14.1 g, 0.04 mole) and acetoxime (4.0 g, 0.055 mole) were dissolved in a mixture of dioxane (160 ml) and AcOEt (40 ml), and dicyclohexylcarbodiimide (DCCD, 9.0 g, 0.044 mole) was added to this solution at 0° with stirring. After stirring for 2 hr at 0°, dicyclohexylurea (DCU) formed was filtered off and the filtrate was concentrated to dryness *in vacuo*. The resulting oily residue was solidified as fine white crystals from MeOH–pet. ether. Recrystallization from MeOH–pet. ether gave the pure ester; 15.3 g (87%), mp 109—112°, $[\alpha]_D^{22}$ −28.0° (c=1.0, in $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O). *Anal.* Calcd. for $C_{17}H_{24}O_6N_6$: C, 49.99; H, 5.92; N, 20.58. Found: C, 49.86; H, 5.93; N, 20.51.

O-(N-Carbobenzoxy-L-alanyl)acetoxime——N-Carbobenzoxy-L-alanine (11.4 g, 0.05 mole) and acetoxime (4.0 g, 0.055 mole) were dissolved in AcOEt (200 ml) and DCCD (10.3 g, 0.05 mole) was added at 0° with stirring. After stirring for 2 hr at 0°, DCU formed was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The oily residue was crystallized by addition of pet. ether and collected by filtration. Recrystallization from AcOEt–pet. ether gave prisms; 12.5 g (89%), mp 60.5—61.5°, $[\alpha]_D^{24}$ +17.0° (c=1.0, in $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O). *Anal.* Calcd. for $C_{14}H_{18}O_4N_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.51; H, 6.54; N, 10.00.

Other acetoxime esters of carbobenzoxy-amino acids and -peptides which were listed in Table I, were prepared in a similar manner.

O-(N-Carbobenzoxyglycyl)cyclopentanonoxime——N-Carbobenzoxyglycine (4.18 g, 0.02 mole) and cyclopentanonoxime (2.07 g, 0.022 mole) were dissolved in a mixture of dioxane (30 ml) and AcOEt (40 ml) and DCCD (4.12 g, 0.02 mole) was added at 0° with stirring. After stirring for 1 hr at 0°, DCU formed was filtered off and the filtrate was concentrated to dryness *in vacuo*, and the oily residue was crystallized by addition of pet. ether. Recrystallization from AcOEt–pet. ether gave needles; 5.40 g (93%), mp 120.5—121.5°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O). *Anal.* Calcd. for $C_{15}H_{18}O_4N_2$: C, 62.05; H, 6.25; N, 9.65. Found: C, 62.36; H, 6.46; N, 9.60.

Other cyclopentanonoxime esters of carbobenzoxy-amino acids which were listed in Table I were prepared in a similar manner.

O-(N-Carbobenzoxy-L-seryl)cyclohexanonoxime——N-Carbobenzoxy-L-serine (9.56 g, 0.04 mole) and cyclohexanonoxime (5 g, 0.044 mole) were dissolved in a mixture of dioxane (50 ml) and AcOEt (50 ml) and DCCD (9 g, 0.044 mole) was added with stirring at 0°. After stirring for 2 hr, DCU formed was filtered off

11) In constract to N-hydroxysuccinimide, the simple ketoximes are soluble in ether and pet. ether.
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22) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, **38**, 1979 (1965).
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24) St. Gutmann and R.A. Boissonnas, *Helv. Chim. Acta*, **199**, 185 (1958).
25) W. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 449 (1958).
26) All the melting points were uncorrected. The completion of the reaction was checked by thin-layer chromatography using Merck's silica gel G and a mixture of $CHCl_3$, MeOH and AcOH (9:1:0.5) as the solvent.

and the filtrate wa sconcentrated to syrup which was soon crystallized as colorless prisms. Recrystallization from AcOEt-pet. ether gave the pure ester; 12.8 g (95.5%), mp 88.0—90.0°, $[\alpha]_D^{23}$ +4.3° (c=1.0, in $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O). *Anal.* Calcd. for $C_{17}H_{22}O_5N_2$: C, 61.06; H, 6.64; N, 8.38. Found: C, 60.86; H, 6.70; N, 8.40.

O-(N-Carbobenzoxy-L-threonyl)cyclohexanonoxime——N-Carbobenzoxy-L-threonine (10.1 g, 0.04 mole) and cyclohexanonoxime (5 g, 0.044 mole) were dissolved in a mixture of dioxane (50 ml) and AcOEt (50 ml) and DCCD (9 g, 0.044 mole) was added at 0°. After stirring for 2 hr at 0°, DCU formed was filtered off and the filtrate was concentrated to syrup which was soon crystallized as colorless prisms. Recrystallization from AcOEt–pet. ether gave the pure ester; 13.2 g (94.0%), mp 91.5—92.5°, $[\alpha]_D^{23}$ −3.5 (c=1.0, in $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (C=O). *Anal.* Calcd. for $C_{18}H_{24}O_5N_2$: C, 62.09; H, 6.94; N, 8.04. Found: C, 62.00; H, 7.02; N, 7.90.

Other cyclohexanonoxime ester of carbobenzoxy-amino acids which were listed in Table I were prepared in a similar manner.

General Procedure for Synthesis of Carbobenzoxy-dipeptide Esters——To a cold solution of an amino acid eater hydrochloride or p-toluenesulphonate (0.01 mole) and triethylamine (1.5 ml) in a solution (20—40 ml) which are listed in Table II, acetic acid (0.2—1.5 eq.) and O-(N-carbobenzoxy-aminoacyl)ketoxime (0.01 mole) were added with stirring. After the reaction was completed, the reaction mixture was concentrated to dryness *in vacuo*, and the resulting residue was dissolved in AcOEt (100 ml). The AcOEt solution which containing the product was washed successively with 1N HCl and H_2O then dried over anhyd. Na_2SO_4. The dried solution was evaporated *in vacuo*, and the crystalline residue was recrystallized from a suitable solvent. The data are given in Table II.

N-Carbobenzoxy-L-nitroarginyl-L-proline Benzyl Ester——To a cold solution of L-proline benzyl ester hydrochloride (4.84 g, 0.02 mole) and triethylamine (3.0 ml) in dioxane (50 ml), AcOH (2.0 ml) and O-(N-carbobenzoxy-L-nitroarginyl)acetoxime (8.2 g, 0.02 mole) were added. After stirring for 24 hr at room temperature, the solvent was evaporated to dryness, and the resulting syrup was crystallized on trituration with H_2O. Recrystallization from MeOH–H_2O gave the pure protected peptide; 9.15 g (97.2%), mp 147—148.5°, $[\alpha]_D^{22}$ −41.3 (c=1.0, in DMF). *Anal.* Calcd. for $C_{26}H_{32}O_7N_6$: C, 57.77; H, 5.97; N, 15.55. Found: C, 57.53; H, 5.85; N, 15.67.

N-Carbobenzoxy-L-threonylglycine Methyl Ester——To a suspension of glycine methyl ester hydrochloride (1.51 g, 0.012 mole) in $CHCl_3$ (30 ml), triethylamine (1.68 ml), AcOH (0.70 ml) and O-(N-carbobenzoxy-L-threonyl)cyclohexanonoxime (3.48 g, 0.01 mole) were added. After stirring for 15 hr at room temperature, the solvent was evaporated off *in vacuo*. The oily residue was dissolved in AcOEt (100 ml), and this AcOEt solution was washed with NaCl-saturated 1N HCl and H_2O, and dried over anhyd. Na_2SO_4. The dried AcOEt solution was evaporated to dryness *in vacuo*. The resulting syrupy residue was crystallized by addition of pet. ether. Recrystallization from AcOEt–pet. ether gave the pure peptide; 2.80 g (86.4%), mp 107.0—109.5°, $[\alpha]_D^{23}$ −15.0° (c=1.0, in MeOH). (lit, [24] mp 105.0—107.0°, $[\alpha]_D^{20}$ −13.9 (c=0.44, in MeOH)). *Anal.* Calcd. for $C_{15}H_{20}O_6N_2$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.69; H, 6.13; N, 8.62.

N-Carbobenzoxyglycyl-L-alanylglycine Ethyl Ester——Carbobenzoxy-L-alanylglycine ethyl ester (2.16 g, 0.007 mole) and AcOH (0.5 ml) were dissolved in 50 ml of EtOH, and hydrogenated over pd-black for 4 hr. After filtration, the filtrate was evaporated to dryness *in vacuo*. The residue and O-(N-carbobenzoxy-glycyl)cyclohexanonoxime (2.13 g, 0.007 mole) were dissolved in 15 ml of dimethylformamide, and the mixture was stirred for 12 hr at room temperature. The reaction mixture was diluted with 80 ml of H_2O and extracted with AcOEt (80 ml×2). The extracted solution was washed with 1N HCl and H_2O, and then dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* to yield a crude crystalline (2.35 g, 90%). Recrystallization from AcOEt–pet. ether gave the pure peptide; 2.15 g (84%), mp 144—145°, $[\alpha]_D^{25}$ −31.3° (c=1.0, in EtOH). (lit, [21] mp 145°). *Anal.* Calcd. for $C_{17}H_{23}O_6N_3$: C, 55.88; H, 6.35; N, 11.50. Found: C, 55.69; H, 6.40; N, 11.56.

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