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Chemical Studies on Amino Acids and Peptides. III.[1] 4,5-Diaryl-4-oxazolin-2-one Derivatives as an Amino Protecting Group

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4,5-Diaryl-4-oxazolin-2-one [V: R=CH_3O (AOX) and R=Cl (COX)] derivatives of amino acids were prepared by condensation of 4,5-diaryl-1,3-dioxol-2-one (III: R=CH_3O and R=Cl) with amino acids. These oxazolinone derivatives were stable to NaOH-aq. EtOH and liquid hydrogen fluoride at room temperature, but were cleaved by hydrogenolysis over pd-carbon. Pentagastrin protected with AOX group, AOX–Gly–Trp–Met–Asp–Phe–NH_2, was prepared and its biological effect was examined on gastric secretion in young chicken.

Keywords——4,5-diaryl-4-oxazolin-2-one derivatives; 4,5-diaryl-1,3-dioxol-2-one; amino protecting group; gastrin pentapeptide; amino acid

4,5-Diphenyl-4-oxazolin-2-one[4] (V: R=H, abbreviated as OX) derivatives have recently been employed as amino protecting groups in peptide synthesis, because this OX group has the following advantages: it is stable to hydrobromic acid—acetic acid and liquid hydrogen fluoride which were used to remove usual protecting groups (Z, BOC group and *etc.*), but is readily deprotected under reductive or oxidative conditions. We now report the preparation of the analogues of this protecting group, 4,5-bis(*p*-methoxyphenyl)-(Va: abbreviated as AOX) and 4,5-bis(*p*-chlorophenyl)-4-oxazolin-2-one (Vb: abbreviated as COX) derivatives of several amino acid in order to examine to the stability of these derivatives under acidic or basic conditions, and also the preparation of pentagastrin protected with AOX group to investigate its pharmacological activity.

As shown in Chart 1, the preparation of AOX and COX derivatives was carried out in the similar manner to the method for synthesis of OX derivatives.[4] 4,5-Diaryl-1,3-dioxol-2-ones, (IIIa) and (IIIb), were prepared by condensation of anisoin (Ia) or 4,4′-dichlorobenzoin (Ib) with phosgene or trichloromethyl chloroformate in the presence of 2.0 equivalent of N,N-dimethylaniline in toluene. Although this condensation would proceed *via* thermal cyclization of unstable chloroformate (II) to 1,3-dioxolone (III) in the same way as the coupling of benzoin with phosgene in benzoin.[4] IIIa was obtained in good yield without heating the corresponding chloroformate, but IIIb in poor yield.

Introduction of protecting group to amino acid was performed by reaction of 1,3-dioxolone, (IIIa) or (IIIb), with the amino acid in the presence of 1.0 equivalent tetramethylammonium hydroxide to furnish hydroxyoxazolidinone (IV) as an intermediate, which, without isolation, was converted into the oxazolone (V) derivatives of amino acid by dehydration with TFA. (Table I.)

1) Part II: M. Kanaoka, T. Ishida, and T. Kikuchi, submitted to *Chem. Pharm. Bull.* (Tokyo), **65**, 605 (1978).
2) Amino acids and their derivatives mentioned in this paper are of L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature; *Biochem.*, **5**, 2485 (1966); *ibid.*, **11**, 1726 (1972). BOC=*tert*-butyloxycarbonyl, DCC=dicyclohexylcarbodiimide, DMF=dimethylformamide, OBzl=benzyl ester, ONP=*p*-nitrophenyl ester, TFA=trifluoroacetic acid, THF=tetrahydrofuran.
3) Location; *3190 Gofuku, Toyama, 930, Japan.*
4) J.C. Sheehan and F.S. Guziec, Jr., *J. Am. Chem. Soc.*, **94**, 6561 (1972); J.C. Sheehan and F.S. Guziec, Jr., *J. Org. Chem.*, **38**, 3034 (1973).

AOX and COX derivatives thus obtained were stable to NaOH–aq. EtOH (at room temperature, 4 hr), liquid hydrogen fluoride (at room temperature, 3 hr), while they were readily deprotected by catalytic hydrogenolysis at room temperature.

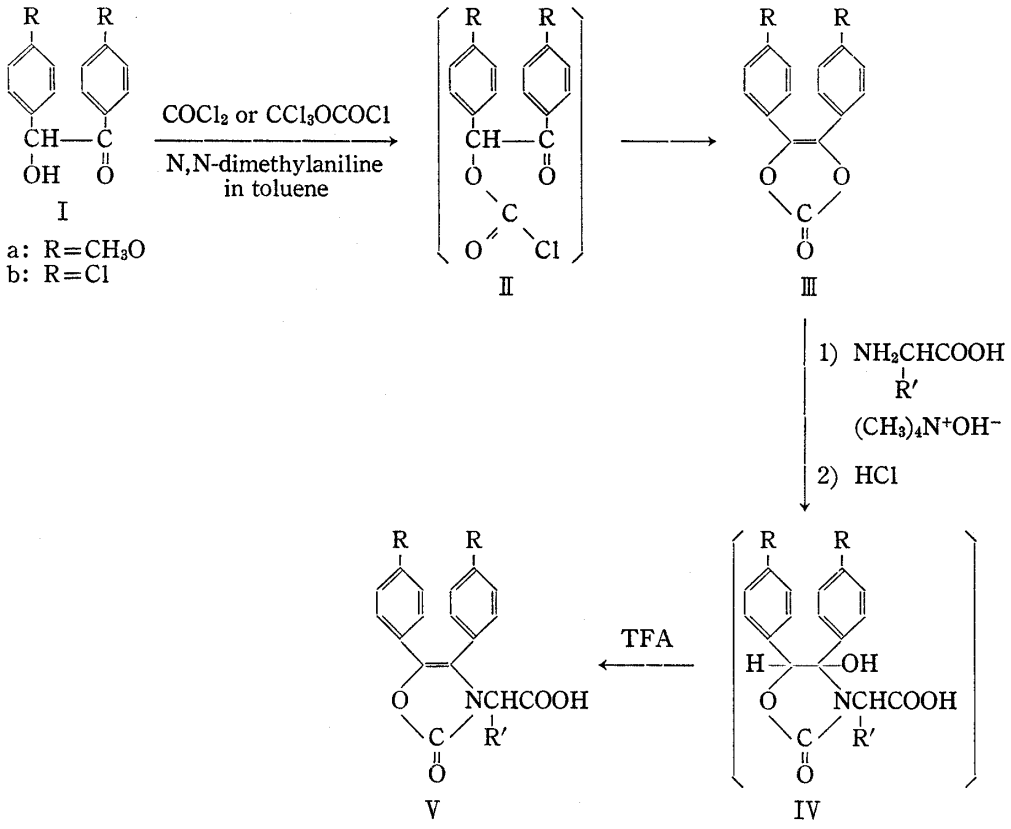


Chart 1

Table I. AOX and COX Amino Acid Derivatives

R	Amino acid	Yield (%)	mp (°C)	$[\alpha]_D^{14}$ (c=1, MeOH)	Formula	Analysis (%) Calad. C	H	N	Found C	H	N
CH_3O	Gly	90	170—172	—	$C_{19}H_{17}NO_6$	64.22	4.82	3.94	64.11	4.78	3.98
CH_3O	Ala	43	219—220	−27.2[a]	$C_{20}H_{19}NO_6$	65.03	5.19	3.79	65.28	4.95	4.03
CH_3O	Phe	82	201—203	−147.0	$C_{26}H_{23}NO_6$	70.10	5.20	3.14	70.03	5.19	3.14
CH_3O	Leu	77	169—170	−22.4	$C_{23}H_{25}NO_6$	67.14	6.12	3.40	66.98	6.04	3.56
Cl	Ala	89	199—200	−27.9[a]	$C_{18}H_{13}Cl_2NO_4$	57.16	3.46	3.70	56.87	3.27	3.53
Cl	Phe	88	224—225	−170[a]	$C_{24}H_{17}Cl_2NO_4$	63.45	3.77	3.08	63.18	3.76	3.26
Cl	Leu	90	119—120	−23.4	$C_{21}H_{19}Cl_2NO_4$	60.00	4.56	3.33	60.22	4.84	3.49

a) At 16°.

Having been reported several methods for the synthesis of pentagastrin,[5] an alternative synthesis of the analogue of this peptide, AOX-Gly-Trp-Met-Asp-Phe-NH_2, using BOC protecting group, is herewith presented as shown in Chart 2. The mixed anhydride condensation

5) *a*) J.C. Anderson, G.W. Kenner, J.K. MacLeod, and R.C. Sheppard, *Tetrahedron, Suppl.* 8, Part 1, **1966**, 39.; *b*) J.M. Davey and J.S. Morley, *J. Chem. Soc.* (*C*), **1966**, 555.; *c*) L. Bernardi, G. Bosisio, R. De Castiglione, and O. Goffredo, *Experientia*, **23**, 700 (1967); *d*) M. Bodanszky and S. Natarajan, *J. Org. Chem.*, **40**, 2495 (1975).

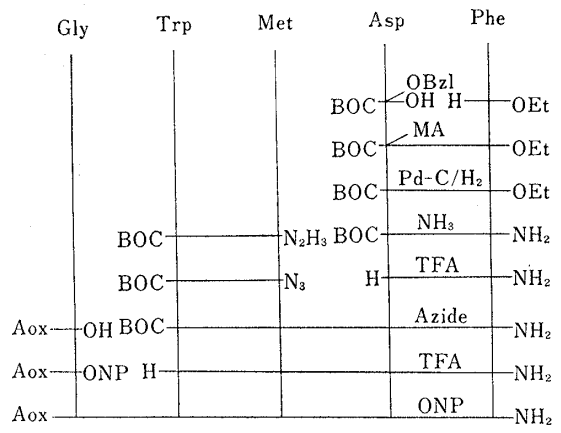


Chart 2

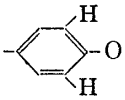
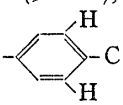
of BOC-Asp(OBzl)-OH and H-Phe-OEt afforded BOC-Asp(OBzl)-Phe-OEt, which after catalytic hydrogenolysis, was treated with ammonia in a usual manner. The resulting dipeptide amide, BOC-Asp-Phe-NH_2, after treatment with TFA, was coupled with the known dipeptide hydrazide, BOC-Trp-Met-$NHNH_2$,[5b] by the azide procedure to give protected tetrapeptide amide, BOC-Trp-Met-Asp-Phe-NH_2. The BOC group of the above tetrapeptide amide was removed by TFA containing anisole under argon atmosphere, and the resulting H-Trp-Met-Asp-Phe-NH_2 trifluoroacetate, after neutralization with triethylamine, was subjected to the next condensation with AOX-Gly-ONP to afford AOX-Gly-Trp-Met-Asp-Phe-NH_2. Bodanszky, *et al.*[5d] reported that the condensation of such peptide containing free side chain of aspartyl residue with BOC-GlyONP in the presence of excess of tertiary amine afforded succinimide dericative as a side product. In case of AOX-Gly-ONP, however, such side reaction was not observed.

In our preliminary experiment it was confirmed that AOX-Gly-Trp-Met-Asp-Phe-NH_2 (150 μg/kg. *s.c.*) showed a potent secretagogue action in anestheized young chicken with acute gastric fistula. The mechanism for the secretagogue action of this compound is now in progress.

Experimental

General experimental methods employed here are essential the same as described in the Part II[1] of this series. Mass spectral (MS) determination were performed with a Nihondenshi JMS-OISG-2 spectrometer with a direct inlet system. Infrared (IR) spectra were measured with a Nihonbunko IRA-2 spectrometer. Nuclear magnetic resonance (NMR) spectra were taken with a Nihondenshi PX-60 spectrometer using tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was performed on Kieselgel G (Merk) with (A) *n*-Hexane–$CHCl_3$ (6:4), (B) pyridine–*n*-BuOH–H_2O (1:2:2 upper phase), (C) $CHCl_3$–MeOH–AcOH (95:5:3). Detection was effected with ultraviolet light or ninhydrin.

4,5-Diaryl-1,3-dioxol-2-one (III)

a) Using Phosgene——Preparation of IIIa: To a stirred solution of anisoin (Ia) (27.2 g, 0.1 mol) in toluene (400 ml) containing phosgene (11 g, 0.11 mol) was added dropwise N,N-dimethylaniline (24.2 g, 0.2 mol) for 1 hr at 0°. After stirring at room temperature for 12 hr, the resulting precipitate was collected by filtration, washed with toluene (30 ml), and dissolved in $CHCl_3$. The solution was washed with H_2O, dried, and then evaporated. The residue was recrystallized from toluene to give IIIa (16 g), mp 175—176°. The above toluene filtrate and washings were combined, and warmed on the water bath at 80—90° for 3 hr. After cooling, the solution was washed with 0.5 N HCl and H_2O, dried and evaporated. The residue was recrystallized from toluene to give IIIa (4 g), mp 175—176°, a sample of which was identified with a sample of the above precipitate by comparision of IR spectra. Total yield was 20 g (67%). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.66; H, 4.66. IR $\nu_{max}^{CCl_4}$ cm^{-1} 1880, 1810 (>C=O), NMR δ (in $CDCl_3$); 3.85 (6H, s, OCH_3), 6.90 (4H, d, J=8 Hz, ) 7.45 (4H, d, J=8 Hz, ). MS *m/e*: 298 (M^+).

Preparation of IIIb: A similar treatment of 4,4′-dichlorobenzoin (7.1 g, 25 mmol) with phosgene (2.8 g, 27 mmol) in the presence of N,N-dimethylaniline (6 g, 50 mmol) gave IIIb (3 g, 40%), mp 141—142°. *Anal.* Calcd. for $C_{15}H_8Cl_2O_3$: C, 58.67; H, 2.61. Found: C, 58.82; H, 2.51. IR $\nu_{max}^{CCl_4}$ cm^{-1} 1810 (>C=O). NMR δ (in $CDCl_3$): 7.4 (8H, m, aromatic H). MS *m/e*: 307 (M^+).

b) Using Trichloromethyl Chloroformate——Preparation of IIIa: To a stirred suspension of anisoin (13.6 g, 0.05 mol) in toluene (150 ml) was added dropwise N,N-dimethylaniline (12.1 g, 0.1 mol) for 1 hr at 0°. By the treatment of the reaction mixture as described (a) gave IIIa (12.2 g, 82%), mp 175—176°.

Preparation of IIIb: Similary, when 4,4′-dichlorobenzoin (7.1 g, 25 mmol) was treated by the same manner as described above, IIIb was obtained in 45% (3.5 g) yield and was mp 141—142°.

4,5-Diaryl-4-oxazolin-2-one Derivatives of Amino Acids (V) (Table I)

General Procedure——To a solution of amino acid (5 mmol) and 10% methanolic tetramethylammonium hydroxide (4.5 g, 5 mmol) in dioxane or DMF (30 ml) was added 1,3-dioxolone (IIIa or IIIb; 5 mmol) and stirred at room temperature for 24—36 hr. The mixture was acidified with 1 N HCl and extracted with AcOEt. The extract was washed with H_2O, dried, and evaporated. The residue was dissolved in TFA (5 ml) and allowed to stand 2 hr at room temperature. Removal of TFA *in vacuo* afforded a pale yellow oil which solidified by trituration with petroleum benzine and recrystallized from AcOEt–petroleum benzin to give V (AOX and COX derivatives of amino acids. Table I).

Stability of AOX and COX Derivatives NaOH——The solution of AOX–Ala–OH (100 mg) or COX–Ala–OH (100 mg) and 4 N NaOH (20 ml) in EtOH (50 ml) was stirred for 4 hr at room temperature. The mixture revealed a single fluorescent spot corresponding to starting material on TLC (B, C). Then mixture was neutralized with 2 N HCl, most of EtOH removed, and the residual solution was acidified with 2 N HCl, extracted with AcOEt. The extract was dried and removed the solvent to afford starting material AOX–Ala–OH (96 mg) and COX–Ala–OH (95 mg), respectively.

HF——A solution of AOX–Ala–OH (100 mg) or COX–Ala–OH (100 mg) in anhydrous liquid hydrogen fluoride (10 ml) was stirred for 3 hr at room temperature. The hydrogene fluorid was removed *in vacuo*, and the residue was dissolved in AcOEt, washed with H_2O and dried. Removal of solvent afforded AOX–Ala–OH (95 mg) and COX–Ala–OH (93 mg), respectively. Each material revealed a single fluorescent spot corresponding to starting material on TLC (B, C).

Catalytic Hydrogenolysis——AOX–Ala–OH (100 mg) or COX–Ala–OH (100 mg) in MeOH (50 ml) containing 1 N HCl (2 ml) was hydrogenated over 10% Pd-carbon (25 mg) until no fluorescent spot was noted on TLC (B, C). The mixture was filtered off and the filtrate evaporated. The residue was recrystallized from EtOH–ether to afford alanine hydrochloride 29 mg (from AOX–Ala–OH), 30 mg (from COX–Ala–OH), respectively.

BOC-Asp(OBzl)-Phe-OEt——To a solution of BOC–Asp(OBzl)–OH (3.3 g, 10 mmol) and N-methylmorpholine (1.1 g, 10 mmol) in THF (30 ml) was added isobutyl chloroformate (1.3 ml, 10 mmol) at −15°. After 3 min, a solution of H–Phe–OEt (prepared from 2.3 g of the hydrochloride and 1.4 ml of Et_3N) in $CHCl_3$ (30 ml) was added to the above reaction mixture at −10°. After addition, the mixture was stirred at −10° for 1 hr and then at room temperature for 24 hr. The mixture, after filtration, was evaporated and the residue was dissolved in AcOEt. The solution was washed successively with 10% citric acid, 5% $NaHCO_3$, dried, and evaporated. The residue was recrystallized from 50% aq. EtOH to give BOC–Asp(OBzl)–Phe–OEt (4 g, 80%), mp 72—73°. *Anal.* Calcd. for $C_{27}H_{34}N_2O_7$: 65.04; H, 6.87; N, 5.62. Found: C, 64.97; H, 6.77; N, 5.68. $[\alpha]_D^{24}$ −9.2 (c=1, EtOH).

BOC-Asp-Phe-OEt——BOC–Asp(OBzl)–Phe–OEt (1 g, 2 mmol) in 80% AcOH (10 ml) was hydrogenated over 10% palladium-carbon (0.1 g) for 5 hr. After removal of catalyst, the solution was evaporated and the residue was recrystallized from 30% aq. MeOH to give BOC–Asp–Phe–OEt (0.7 g, 86%), mp 133—134°. *Anal.* Calcd. for $C_{20}H_{28}N_2O_7$: C, 58.81; H, 6.91; N, 6.86. Found: C, 58.63; H, 6.70; N, 6.87. $[\alpha]_D^{24}$ −9.2 (c=1, EtOH).

BOC-Asp-Phe-NH_2——BOC–Asp–Phe–OEt (0.82 g, 2 mmol) and 25% NH_3–MeOH (30 ml) were stirred at room temperature 24 hr. The mixture was evaporated, and the residue was acidified with 10% citric acid and extracted with AcOEt, dried, and evaporated. Recrystallization of the residue from EtOH–petroleum benzine gave BOC–Asp–Phe–NH_2 (0.6 g, 79%), mp 205—206° (dec.). *Anal.* Calcd. for $C_{18}H_{25}N_3O_6$: C, 56.98; H, 6.64; N, 11.08. Found: C, 56.97; H, 6.59; N, 10.91. $[\alpha]_D^{24}$ −44.4 (c=1.47, DMF).

BOC-Trp-Met-Asp-Phe-NH_2——BOC–Asp–Phe–NH_2 (0.3 g, 1 mmol) was treated with TFA in an ice bath for 30 min. Dry ether was added and the resulting powder was collected by filtration, washed with dry ether, dried over KOH pellets *in vacuo* for 5 hr and then dissolved in DMF (2 ml). To this ice-chilled solution, Et_3N (0.14 ml, 1 mmol) and the azide [prepared from BOC–Trp–Met–$NHNH_2$[5b] (0.45 g, 1 mmol) with 1.7 N HCl–THF (1.2 ml, 2 mmol), isoamylnitrite (0.15 ml, 1 mmol) and Et_3N (0.28 ml, 2 mmol)] in THF (2 ml) were added. The mixture was stirred at 0° for 48 hr, the solvent was evaporated and the residue was treated 10% citric acid and ether. The resulting powder was recrystallized from 50% aq. EtOH to give BOC–Trp–Met–Asp–Phe–NH_2 (0.45 g, 65%), mp 209—210° (dec.). $[\alpha]_D^{24}$ −35.0 (c=1, DMF) (lit[5d] mp 212—213° (dec.), $[\alpha]_D^{25}$ −35.7 (c=1, DMF). *Anal.* Calcd. for $C_{34}H_{44}N_6O_8S$: C, 58.60; H, 6.37; N, 12.06. Found: C, 58.54; H, 6.30; N, 12.02.

AOX-Gly-ONP——To a stirred solution of AOX–Gly–OH (1.8 g, 5 mmol) and *p*-nitrophenol (0.7 g, 5 mmol) in THF (30 ml) was added a solution of DCC (1.1 g, 5 mmol) in THF (10 ml) at 0°. The resulting precipitate was filtered off, and the filtrate was evaporated. The residue was dissolved in AcOEt, which was washed with 5% $NaHCO_3$, dried, and evaporated. The residue was recrystallized from MeOH to give AOX–Gly–ONP (2.1 g, 88%), mp 125—126°. *Anal.* Calcd. for $C_{25}H_{20}N_2O_8$: C, 63.02; H, 4.23; N, 5.88. Found: C, 63.03; H, 4.22; N, 5.63.

AOX-Gly-Trp-Met-Asp-Phe-NH_2——BOC–Trp–Met–Asp–Phe–NH_2 (0.24 g, 0.34 mmol) was treated with TFA containing 5% anisole under argon atmosphere as stated above. The resulting TFA salt as a crystalline by dry ether was dissolved in DMF (3 ml) containing Et_3N (0.1 ml). To this solution was added a solution of AOX–Gly–ONP (0.19 g, 0.4 mmol) in DMF (1.5 ml). The mixture was stirred at room temperature for

48 hr and the solvent was evaporated and the residue was treated with AcOEt and ether. The resulting powder was precipitated twice from DMF with ether to give AOX–Gly–Trp–Met–Asp–Phe–NH_2 (0.23 g, 72%), mp 240—242°, $[\alpha]_D^{16}$ −12.4 (c=1.03, DMF). *Anal.* Calcd. for $C_{48}H_{51}N_7O_{11}S$: C, 61.72; H, 5.50; N, 10.50. Found: C, 61.61; H, 5.56; N, 10.34.

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