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Chemical Studies on Amino Acids and Peptides. II.[1] Syntheses of Choline Esters of Peptides related to Tetragastrin[2]

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Choline esters of peptides related to tetragastrin, Z–Asp(OBu^t)–Phe–OCh$^+$ Br$^-$ (XII), Z–Met–Asp(OBu^t)–Phe–OCh$^+$ Br$^-$ (XIII), Z–Trp–Met–Asp(OBu^t)–Phe–OCh$^+$ Br$^-$ (XIV), and BOC–Trp–Met–Asp(OBu^t)–Phe–OCh$^+$ Br$^-$ (XV) were synthesized by the condensation of N-protected peptides, (VI), (VII), (VIII), and (IX), with β-dimethylaminoethyl bromide followed by methylation with methyl bromide. Selective removal of the protective groups from the above peptide choline esters was achieved by conventional acid treatment under mild conditions.

Keywords——peptide choline ester; esterification of peptides; tetragastrin; smooth muscle contraction; acetylisoleucine choline ester

Recently, a smooth muscle contracting substance was isolated from the water-soluble fraction of a Chinese drug "Benzoar Oriental" by Kimura, *et al.*[4] who suggested on the basis of pharmacological study that the substance is probably a peptide-like one containing choline ester grouping. Although a number of biologically important peptides have been isolated from natural resources and numerous studies on their chemical and pharmacological behaviors have been published, no report on the peptide choline ester could be found in literature. We were interested in this particular class of compounds and commenced a synthetic study in order to examine their pharmacological activities. In the preceding paper,[1] we reported the preparation of several L-α-amino acid choline esters whose potency of smooth muscle contraction was found to be intermediate between those of acetylcholine and choline.

Among naturally occurring peptides, gastrin, a typical gastrointestinal hormone, and its related peptides were reported to show the smooth muscle contracting activity.[5] In this connection, we now synthesized choline esters of peptides related to tetragastrin[6] (gastrin C-terminal tetrapeptide) in which C-terminal amide linkages were replaced by choline ester linkage and the result is described herewith.

Preparation of Peptides Related to Tetragastrin

As shown in Chart 1, Z-Asp(OBu^t)-Phe-OEt (I) and Z-Met-Asp(OBu^t)-Phe-OEt (III) were synthesized by mixed anhydride method using ethyl chloroformate.

1) Part I: M. Kanaoka and M. Kimura, *Yakugaku Zasshi*, **95**, 231 (1975). Part of this work was presented at the 42nd Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, June 1976.
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). Ac=acetyl, BOC=*tert*-butyloxycarbonyl, OCh$^+$=$-OCH_2CH_2N^+(CH_3)_3$, $-OBu^t$=*tert*-butoxy, TFA=trifluoroacetic acid, THF=tetrahydrofuran, Tos=*p*-toluenesulfonyl and Z=benzyloxycarbonyl.
3) Location: *3190 Gofuku, Toyama, 930, Japan.*
4) M. Kimura, E. Osada, T. Honke, M. Yoshizaki, and D. Shiho, *Yakugaku Zasshi*, **86**, 877 (1966); M. Kimura and M. Kanaoka, Proceedings of Symposium on WAKAN-YAKU, **6**, 122 (1972).
5) E.S. Vizi, G. Bertaccini, M. Impicciatore, and J. Knoll, *European J. Pharm.*, **17**, 175 (1972).
6) H.J. Tracy and R.A. Gregory, *Nature* (London), **204**, 935 (1964); A. von Dungen, W. Konz, and H. Hummelt, *Ann. Chem.*, **1976**, 860.

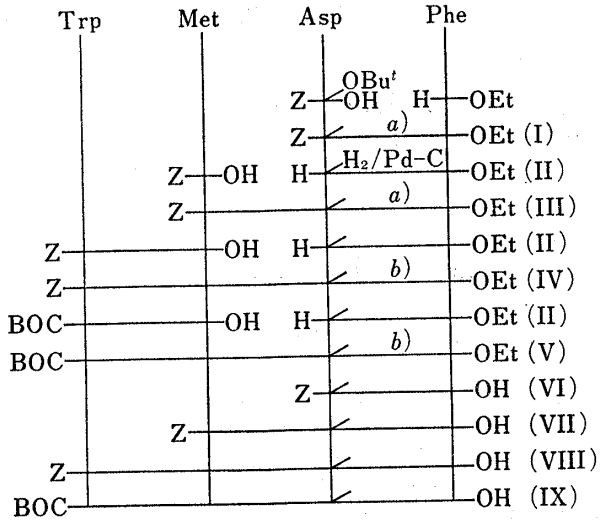

Chart 1

a) ethyl chloroformate+Et_3N.
b) iso-butyl chloroformate+N-methylmorpholine.

For the preparation of Z-Trp-Met-Asp(OBu^t)-Phe-OEt (IV), usual stepwise elongation using the above tripeptide (III) would be unpromising, because the removal of Z group from III by catalytic hydrogenolysis is generally difficult due to the presence of N-terminal methionine residue.[7] Therefore, the tetrapeptide (IV) was prepared by the coupling of Z-Trp-Met-OH and H-Asp(OBu^t)-Phe-OEt (II) according to the method of Anderson, *et al.*[8] Furthermore, BOC-Trp-Met-Asp-(OBu^t)-Phe-OEt (V) was also prepared from BOC-Trp-Met-OH in the similar manner, which was conveniently employed for the synthesis of unprotected tetrapeptide choline ester (XXI) (see Table II (C)).

The above peptide ethyl esters (I, III, IV, and V) were then hydrolyzed in the usual manner to afford Z-Asp(OBu^t)-Phe-OH (VI), Z-Met-Asp-(OBu^t)-Phe-OH (VII), Z-Trp-Met-Asp(OBu^t)-Phe-OH (VIII), and BOC-Trp-Met-Asp-(OBu^t)-Phe-OH (IX), respectively.

Esterification of the protected peptides was carried out with β-dimethylaminoethyl halide according to the method reported previously.[1] Since a peptide or acylamino acid generally undergoes racemization more easily than Z-amino acid, Bodanszky-Conklin's procedure[9] was applied to examine the racemization which might have occurred in the C-terminal amino acid moiety during the esterification of protected peptides.

Reaction of Ac-Ile-OH (X)[10] with β-dimethylaminoethyl chloride or bromide in ethyl acetate in the presence of triethylamine followed by treatment with methyl *p*-toluenesulfonate gave Ac-Ile-OCh$^+$ TosO$^-$(XI). Degree of racemization of the isoleucine residue in XI was given in Table I, which indicated that virtually no racemization had taken place in the prepa-

Table I. Degree of Racemization during Synthesis of Ac-Ile-OCh$^+$ TosO$^-$

$$\text{Ac-Ile-OH (X)} \xrightarrow[\text{2) } TosOCH_3]{\text{1) } X\text{-}CH_2CH_2N(CH_3)_2 + Et_3N,\ X=Cl, Br} \text{Ac-Ile-OCh}^+\text{TosO}^- \text{ (XI)}$$

Compounds	Degree of racemization[a)]
Ac-Ile-OH	1.2
Ac-Ile-OCh$^+$ TosO$^-$[b)]	1.2
Ac-Ile-Och$^+$ TosO$^-$[c)]	1.4

a) (100×D-aIle-OH)/(Ile-OH+D-aIle-OH).
b) Prepared from β-dimethylaminoethyl chloride.
c) Prepared from β-dimethylaminoethyl bromide.

7) *a*) M. Bodanszky, Y.S. Klausner, and M.A. Ondetti, "Peptide Synthesis," Vol. 1, John Wiley and Sons, Inc., New York, 1976, p. 184; *b*) Yajima, *et al.* reported examples of such catalytic hydrogenolyses of Z-peptides having C-terminal methionine residue. See H. Yajima, K. Kawasaki, Y. Kinomura, T. Oshima, S. Kimoto, and M. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1342 (1968).
8) G.W. Anderson, J.E. Zimmerman, and F.M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012 (1967).
9) M. Bodanszky and L.E. Conklin, *Chem. Commun.*, **1967**, 773.
10) W.A.H. Huffman and A.W. Ingersoll, *J. Am. Chem. Soc.*, **73**, 3366 (1951); J.P. Greenstein, L. Levintow, C.G. Baker and J. White, *J. Biol. Chem.*, **188**, 647 (1951).

Table II

	mp (°C)	$[\alpha]_D^{19°}$ (conc.)	Yield (%)	Appearance (Colorless)	Formula	Analysis (%) Calcd. (Found) C	H	N	PPC *Rf* (Solvent)
(A) Z-Peptide-OBu^t-OH $\xrightarrow[2)\ CH_3Br]{1)\ BrCH_2CH_2N(CH_3)_2+Et_3N}$ Z-Peptide-OBu^t-OCh$^+$Br$^-$									
Z-Asp(OBu^t)-Phe-OCh$^+$Br$^-$ (XII)	81	−18.3(1.6)	75	Needles	$C_{30}H_{42}BrN_3O_7$	56.60 (56.41)	6.65 (6.91)	6.60 (6.34)	0.52(A)
Z-Met-Asp-(OBu^t)-Phe-OCh$^+$Br$^-$ (XIII)	74—76	−18.7(1.1)	77	Prisms	$C_{35}H_{51}BrN_4O_8S$	54.75 (54.94)	6.70 (6.60)	7.30 (7.35)	0.62(A)
Z-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$Br$^-$ (XIV)	89—91	−10.8(1.0)	73	Prisms	$C_{46}H_{61}BrN_6O_9S$	57.91 (57.64)	6.45 (6.68)	8.81 (8.75)	0.77(B)
BOC-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$Br$^-$ (XV)	114—116	−18.2(1.1)	72	Prisms	$C_{43}H_{63}BrN_6O_9S$	56.14 (56.30)	6.90 (6.55)	9.14 (9.46)	0.87(B)
(B) Z-Peptide-OBu^t-OCh$^+$Br$^-$ $\xrightarrow{TFA+anisole}$ Z-Peptide-OCh$^+$Br$^-$									
Z-Asp-Phe-OCh$^+$Br$^-$ (XVI)	84—85	−16.7(1.0)	87	Prisms	$C_{26}H_{34}BrN_3O_7$	53.80 (53.77)	5.90 (5.98)	7.24 (7.11)	0.46(A)
Z-Met-Asp-Phe-OCh$^+$Br$^-$ (XVIII)	85—87	−18.2(1.0)	84	Needles	$C_{31}H_{43}BrN_4O_8S$	52.32 (52.05)	6.09 (6.28)	7.87 (7.63)	0.54(A)
Z-Trp-Met-Asp-Phe-OCh$^+$Br$^-$ (XVIII)	118—120	−8.7(1.2)	82	Prisms	$C_{42}H_{53}BrN_6O_9S$	56.18 (56.11)	5.95 (5.88)	9.39 (9.40)	0.71(B)
(C) Z-Peptide-OCh$^+$Br$^-$ $\xrightarrow{HBr-AcOH+anisole}$ H_2^+-Peptide-OCh$^+$2Br$^-$									
H_2^+-Asp-Phe-OCh$^+$2Br$^-$ (XIX)	126—127	−7.5(2.2)	75	Needles	$C_{18}H_{29}Br_2N_3O_5$	41.00 (41.04)	5.54 (5.84)	7.97 (7.76)	0.24(A)
H_2^+-Met-Asp-Phe-OCh$^+$2Br$^-$ (XX)	144—146	−12.2(1.2)	75	Needles	$C_{23}H_{38}Br_2N_4O_6S$	41.95 (41.66)	5.82 (6.11)	8.51 (8.31)	0.31(A)
H_2^+-Trp-Met-Asp-Phe-OCh$^+$Br$^-$ CF_3COO^- (XXI)[a]	126—128	−10.8(0.9)	77	Prisms	$C_{36}H_{48}BrF_3N_6O_9S$	49.26 (49.60)	5.51 (5.52)	9.57 (9.36)	0.55(B)

a) Prepared from XV by treating with TFA in the presence of anisole.

ration of choline esters and also there was no significant difference between the reactions with β-dimethylaminoethyl chloride and β-dimethylaminoethyl bromide. Thus the more reactive bromide was employed thereafter.

The protected peptides, (VI), (VII), (VIII), and (IX) were allowed to react with β-dimethylaminoethyl bromide in the presence of triethylamine to give the corresponding dimethylaminoethyl esters and the latter were treated with methyl bromide to give Z-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$ (XII), Z-Met-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$(XIII), Z-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$ (XIV), and BOC-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$ (XV), respectively, in satisfactory yields as shown in Table II (A).

Removal of the *tert*-butoxyl group from these protected peptide choline esters, (XII), (XIII), and (XIV), was achieved by the reaction with trifluoroacetic acid in the presence of anisole to give Z-Asp-Phe-OCh$^+$ Br$^-$ (XVI), Z-Met-Asp-Phe-OCh$^+$ Br$^-$ (XVII), and Z-Trp-Met-Asp-Phe-OCh$^+$ Br$^-$ (XVIII), respectively (Table II (B)). Simultaneous removal of the Z and *tert*-butoxyl group from XII and XIII was performed by treating them with hydrobromic acid–acetic acid in the presence of anisole to furnish H_2^+-Asp-Phe-OCh$^+$ 2Br$^-$ (XIX) and H_2^+-Met-Asp-Phe-OCh$^+$ 2Br$^-$ (XX), respectively.

In the case of Z-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$ (XIV), however, undesired decomposition[11] of the tryptophan residue would occur with hydrobromic acid and hence we used BOC-Trp-Met-Asp(OBu^t)-Phe-OCh$^+$ Br$^-$ (XV) instead of XIV. Reaction of XV with trifluoroacetic acid in the presence of anisole gave H_2^+-Trp-Met-Asp-Phe-OCh$^+$ Br$^-$ CF_3COO^- (XXI) in 77% yield as shown in Table II (C).

Pharmacological investigation of these peptide choline esters is now in progress and the result will be published elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Paper partition chromatography (PPC) was performed on Toyo Filter Paper No. 51 by ascending method with (A) *n*-BuOH–AcOH–H_2O (60:12:25) and (B) *n*-BuOH–AcOH–H_2O (4:1:5). Thin-layer chromatography (TLC) was performed on Kieselgel G with $CHCl_3$–MeOH–AcOH (95:5:3). Detection of spots was effected with ninhydrin or Dragendorff reagent. Optical rotations were determined in ethanol solutions with a Nihonbunko DIP-4 polarimeter. Amino acid analyses were carried out on a Nihondenshi JLC-6AH amino acid analyzer. Organic solutions were dried over anhydrous sodium sulfate and evaporations were carried out below 40° under reduced pressure unless otherwise specified.

Z-Asp(OBu^t)-Phe-OEt (I)——To a stirred solution of Z–Asp(OBu^t)–OH[12] (13 g, 40 mmol) and Et_3N (5.6 ml, 40 mmol) in THF (80 ml) was added ethyl chloroformate (3.6 ml, 40 mmol) at −15°. After 3 min, a solution of H–Phe–OEt (prepared from 9.2 g of the hydrochloride and 5.6 ml of Et_3N) in $CHCl_3$ (120 ml) was added to the above reaction mixture under vigorous stirring and the stirring continued for 2 hr at −10° and then for 16 hr at room temperature. The mixture, after filtration, was evaporated and the residue was dissolved in AcOEt, washed successively with 10% aq. citric acid, sat. NaCl, and 5% aq. $NaHCO_3$. Drying followed by evaporation afforded I as a colorless oil (9 g, 86%) which revealed single spot on TLC and was used directly for the next step.

H-Asp(OBu^t)-Phe-OEt (II)——Compound I (7.47 g, 15 mmol) in MeOH (80 ml) containing 1 N HCl (6 ml) was hydrogenated over 10% Pd–carbon (1.0 g) for 5 hr. Filtration and evaporation gave II·HCl as a colorless oil (6.0 g, quantitative), which revealed single spot on TLC and was used directly for the next step.

Z-Asp(OBu^t)-Phe-OH (VI)——To a solution of I (2.5 g, 5 mmol) in MeOH (10 ml) was added 1 N NaOH (6 ml) and the mixture was stirred at room temperature for 45 min. After neutralization with HCl under ice-cooling, most of MeOH was evaporated at 25° and the residual solution was acidified with HCl and extracted with AcOEt. Usual working up of the extract gave a colorless oil (2.1 g, 87%), which was dissolved in AcOEt and treated with dicyclohexylamine to afford the crystalline dicyclohexylammonium salt. Recrystallizations from EtOH–AcOEt gave colorless needles, mp 114—115°. *Anal.* Calcd. for $C_{37}H_{53}N_3O_7$: C, 68.18; H, 8.20; N, 6.44. Found: C, 67.99; H, 8.25; N, 6.23.

11) E. Schröder and K. Lübke, "The Peptides," Academic press., New York, 1965, p. 29.
12) M. Itoh, *Chem. Pharm. Bull.* (Tokyo), **17**, 1679 (1969).

Z-Met-Asp(OBu^t)-Phe-OH (VII)——To a stirred solution of Z–Met–OH (2.9 g, 10 mmol) and Et_3N (1.4 ml, 10 mmol) in THF (30 ml) was added ethyl chloroformate (0.9 ml, 10 mmol) at −15°. After 3 min, a chilled solution of II (prepared from 4.2 g of the hydrochloride and 1.4 ml of Et_3N) in $CHCl_3$ (30 ml) was added to the above mixture and the mixture was stirred at −10° for 2 hr and at room temperature for 24 hr. The mixture was worked up in the same manner as described for I to give Z–Met–Asp(OBu^t)–Phe–OEt (III) as colorless needles (4.7 g, 76%), mp 116—117°. *Anal.* Calcd. for $C_{32}H_{43}N_3O_8S$: C, 61.03; H, 6.88; N, 6.67. Found: C, 61.07; H, 7.00; N, 6.68.

Compound III (3.3 g) was hydrolyzed with NaOH–aq. MeOH in the same manner as described for VI. After usual working-up, the crude product was recrystallized from EtOH–petroleum benzine to give VII (2.6 g, 83%) as colorless needles, mp 68—70°. *Anal.* Calcd. for $C_{30}H_{39}N_3O_8S$: C, 59.88; H, 6.53; N, 6.98. Found: C, 59.59; H, 6.78; N, 6.77.

Z-Trp-Met-Asp(OBu^t)-Phe-OH (VIII)——Z–Trp–Met–OCH_3[13] (3.2 g, 6.6 mmol) was hydrolyzed with NaOH (0.3 g, 8 mmol) in aq. acetone (1: 1, 16 ml) at room temperature in the usual manner to give Z–Trp–Met–OH (2.4 g, 5.2 mmol) as a colorless oil, which was dissolved in THF (20 ml) and chilled to −15°. To this solution was added N-methylmorpholine (0.51 g, 5 mmol) and isobutyl chloroformate (0.65 ml, 5 mmol) under stirring. After 1 or 2 min, a solution of II (prepared from 2.1 g of the hydrochloride and 0.7 ml of Et_3N) in $CHCl_3$ (15 ml) was added to the above mixture and the stirring continued for 2 hr at −10° and then for 16 hr at room temperature. The mixture was worked up in the same manner as described for I to give Z–Trp–Met–Asp(OBu^t)–Phe–OEt (IV) as a colorless oil which was used directly for the next step.

The above compound IV (2.1 g) was hydrolyzed carefully with NaOH–aq. MeOH in the same manner as stated above to yield the corresponding acid (VIII) as a syrup (1.8 g, 90%). Dicyclohexylammonium salt of VIII crystallized in colorless needles from EtOH–AcOEt, mp 94°. *Anal.* Calcd. for $C_{53}H_{72}N_6O_9S$: C, 65.68; H, 7.49; N, 8.96. Found: C, 65.49; H, 7.55; N, 8.42.

BOC-Trp-Asp(OBu^t)-Phe-OH (IX)——BOC–Trp–Met–OCH_3[14] was hydrolyzed with NaOH (1.2 equivalent) in aq. acetone in a similar manner as described for VI. To a stirred solution of BOC–Trp–Met–OH (2.26 g, 5.2 mmol) and N-methylmorpholine (0.51 g, 5 mmol) in THF (50 ml) was added isobutyl chloroformate (0.65 ml, 5 mmol) at −15°. After 1 or 2 min, a solution of II (prepared from 2.1 g of the hydrochloride and 0.7 ml of Et_3N) in $CHCl_3$ (20 ml) was added to the above mixture and the mixture was stirred at −10° for 2 hr and then at room temperature for 16 hr. Usual working up of the reaction mixture gave a colorless oil which solidified on trituration with petroleum benzine. Recrystallization from EtOH–ether afforded BOC–Trp–Met–Asp(OBui)–Phe–OEt (V) (3.1 g, 80%), colorless needles, mp 83°. *Anal.* Calcd. for $C_{40}H_{55}N_5O_9S$: C, 61.44; H, 7.09; N, 8.96. Found: C, 61.15; H, 7.31; N, 8.86.

Compound V (1.95 g) was hydrolyzed with NaOH–aq. MeOH in the same manner as described for VI to give IX (1.7 g, 90%) as a colorless oil, which revealed single spot on TLC and was used directly for the next step.

Bodanszky-Conklin's Test——i) Ac–Ile–OCh$^+$ TosO$^-$ **(XI)**: To a solution of Ac–Ile–OH[10] (1.73 g, 10 mmol) in AcOEt (30 ml) was added Et_3N (1.4 ml, 10 mmol) and 2 M solution of β-dimethylaminoethyl chloride in benzene (7.5 ml, 15 mmol). The mixture was refluxed for 5 hr and then filtered. The filtrate was washed successively with 5% aq. $NaHCO_3$ and sat. aq. NaCl, dried, and evaporated to afford a syrup which was dissolved in AcOEt and methyl *p*-toluenesulfonate (2.5 g) was added. On standing the mixture in a refrigerator, a crystalline solid deposited which was recrystallized from EtOH to give colorless prisms (XI) in 88% yield, mp 184—186°, $[\alpha]_D^{20}$ −8.3 (c=2.0). *Anal.* Calcd. for $C_{20}H_{34}N_2O_6S$: C, 55.79; H, 7.96; N, 6.51. Found: C, 55.82; H, 7.86; N, 6.70.

The same reaction using β-dimethylaminoethyl bromide instead of the chloride gave rise to the same product (XI) in 90% yield, mp 183—185°, $[\alpha]_D^{20}$ −8.5 (c=2.0).

ii) Amino Acid Analysis: Ac–Ile–OH and Ac–Ile–OCh$^+$TosO$^-$ were hydrolyzed with "constant-boiling hydrochloric acid" in evacuated, sealed tubes at 110° for 16 hr, respectively, and the hydrolyzates were submitted to amino acid analyses. The result was shown in Table I.

Preparation of Protected Peptide Choline Esters (Table II (A)). General Procedure——A solution of each protected peptide (VI, VII, VIII, and IX; 3 mmol) in AcOEt (20 ml), 2 M β-dimethylaminoethyl bromide in benzene (2.5 ml, 5 mmol), and Et_3N (0.4 ml, 3 mmol) were combined and refluxed for 3 hr. The mixture, after filtration, was washed successively with 5% aq. $NaHCO_3$ and sat. aq. NaCl, dried, and evaporated. The residue was dissolved in AcOEt (3 ml) and to this solution was added 1.2 M solution of methyl bromide in benzene (3 ml, 3.6 mmol) and the solution was left in a refrigerator. The resulted crystalline solid was recrystallized from EtOH–ether to give the corresponding choline ester bromide.

Removal of *tert*-Butoxyl Group of Protected Peptide Choline Esters (Table II (B)).——A mixture of each protected peptide choline ester (XII, XIII, and XIV; 0.05 mmol), anisole (0.1 ml), and TFA (0.3 ml) was stirred at room temperature for 30 min under argon atmosphere and then the mixture was diluted with

13) H. Gregory, A.H. Laird, J.S. Morley, and J.M. Smith, *J. Chem. Soc.* (*C*), **1968**, 522.
14) J.M. Davey, A.H. Laird, and J.S. Morley, *J. Chem. Soc.* (*C*), **1966**, 555.

ether to precipitate an oily product. This product (XVI, XVII and XVIII) solidified readily upon trituration with petroleum benzine and it was recrystallized from ether–EtOH.

Removal of Z Group and *tert*-Butoxyl Group from Protected Peptide Choline Esters (Table II (C))——A cooled mixture of each protected peptide choline ester (XII and XIII; 0.05 mmol), anisole (0.15 ml), and 25% HBr–AcOH (0.3 ml) was vigorously stirred under argon atmosphere in an ice bath for 30 min and then at room temperature for additional 30 min. The mixture was diluted with ether and the resulted precipitate was recrystallized from EtOH–ether to give XIX and XX, respectively.

H_2^+-Trp-Met-Asp-Phe-OCh^+ Br^- CF_3COO^- (XXI)——A mixture of XV (27 mg, 0.03 mmol), anisole (0.1 ml), and TFA (0.3 ml) was vigorously stirred under argon atmosphere in an ice bath for 30 min and then at room temperature for 30 min. On addition of ether to the reaction mixture, there was obtained a precipitate which was recrystallized from ether–EtOH to give XXI.

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