was distilled *in vacuo* to give 120 mg of X as colorless oil (solidified on standing), bp 120—130° (bath temp., 1 mmHg). IR ν_{max}^{film} cm^{-1}: 2780 (NMe), 1685 (Ar–C=O). NMR ($CDCl_3$) τ: 7.40 (s, 3, NMe), 2.83—2.45 (m, 3, C-8, C-9, C-10 H), 2.01 (a pair of d, $J_{7,8}$=7.5 Hz, $J_{7,9}$=2.0 Hz, 1, C-7 H). Mass Spectrum *m/e*: 201 (M^+). Picrate: mp 245—247° (from MeOH). *Anal.* Calcd. for $C_{19}H_{18}O_8N_4$: C, 53.02; H, 4.22; N, 13.02. Found: C, 53.09; H, 4.23; N, 12.75.

3-Methyl-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine (XIb)——Amino ketone (X) (126 mg) was hydrogenated over Pd-C (40%, 70 mg) in EtOH (6 ml) and AcOH (2 ml) for 2 hr. After removal of the catalyst and solvent, the residual syrup was dissolved in water, basified with 10% NaOH, extracted with ether, and dried (K_2CO_3). The residue of the ethereal solution was distilled *in vacuo* to give 98 mg of XIa as a colorless oil, bp 135—140° (bath temp., 1 mmHg). IR ν_{max}^{film} cm^{-1}: 3440 (OH), 2790 (NMe). NMR ($CDCl_3$) τ: 7.92 (s, 3, NMe), 5.12 (d, J=7.5 Hz, 1, C-6 H). Picrate: mp 222—224° (from MeOH). *Anal.* Calcd. for $C_{19}H_{20}O_8N_4$: C, 52.78; H, 4.66; N, 12.96. Found: C, 52.91; H, 4.67; N, 12.65.

Compound (XIa) (56 mg), 57% HI (2 ml), P (red, 20 mg), AcOH (4 ml) and H_2O (1 ml) were refluxed for 4 hr. The cooled mixture was filtered and evaporated. The brown residue was dissolved in water, basified with 10% NaOH, extracted with ether and dried (K_2CO_3). Evaporation of the solvent gave 48 mg of XIb as a colorless oil, distilled at 95—100° (bath temp., 1 mmHg). IR ν_{max}^{film} cm^{-1}: 2780 (NMe). NMR ($CDCl_3$) τ: 7.92 (s, 3, NMe), 2.95 (s, 4, arom. H). Picrate: mp 246—248° (from MeOH). *Anal.* Calcd. for $C_{19}H_{20}O_7N_4$: C, 54.80; H, 4.84; N, 13.46. Found: C, 54.88; H, 4.82; N, 13.23.

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Studies on Peptides. LVIII.[1,2] Synthesis of Tyr^1-Substance P

HARUAKI YAJIMA, KOUKI KITAGAWA,[3a] TOMIO SEGAWA, and YOSHIHIRO NAKATA[3b]

Faculty of Pharmaceutical Sciences, Kyoto University[3a] and Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine[3b]

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In order to obtain a useful compound for the radioimmunoassay of substance P, Tyr^1-substance P was synthesized by the conventional method.

Since the complete amino acid sequence of substance P from bovine hypothalami and horse intestine was determined by Leeman, *et al.*[4] and Studer, *et al.*[5] respectively, this important physiological principle was synthesized by the conventional method[6,7] as well as by the solid phase method.[8–10] As far as the conventional method is concerned, we recorded the first synthesis of the undecapeptide amide corresponding to the entire amino acid sequence of substance P. We wish to record further the synthesis of a useful derivative for its radio-

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2) Amino acids, peptides and their derivatives are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, DCC=dicyclohexylcarbodiimide, DMF=dimethylformamide, TFA=trifluoroacetic acid.
3) Location: *a) Sakyo-ku, Kyoto, 606, Japan; b) Kasumi-cho, Hiroshima, 734, Japan.*
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immunoassay, named Tyr^1-substance P. At present, [8-Tyr]-substance P is the only synthetic analogue prepared for this purpose.[11]

Available two intermediates in our previous synthesis of substance P were utilized to prepare this parent tracer compound as shown in Fig. 1. Z–Arg(NO_2)–Pro–OH[6] was treated with hydrogen bromide in acetic acid and the resulting H–Arg(NO_2)–Pro–OH was condensed with Z(OMe)–Tyr–$NHNH_2$[12] by the modified azide procedure[13] to give Z(OMe)–Tyr–Arg(NO_2)–Pro–OH, which was purified by the usual extraction procedure followed by precipitation from ethyl acetate with ether.

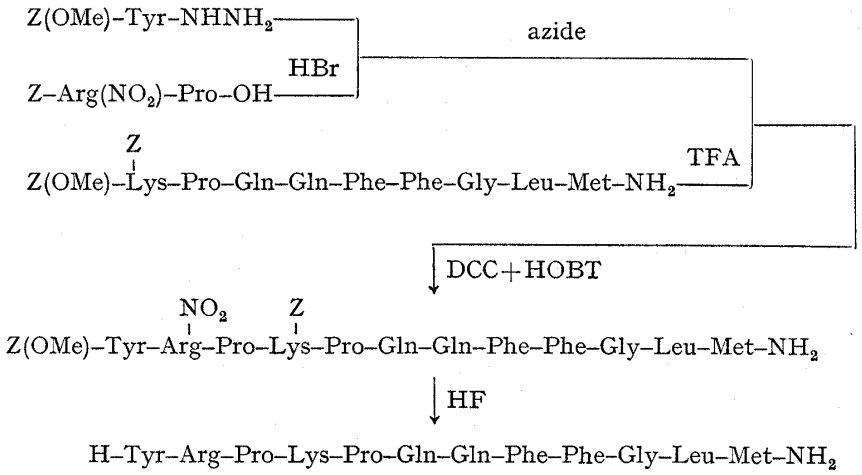

Fig. 1. Synthetic Scheme of Tyr^1-substance P

This protected tripeptide was then condensed with H–Lys(Z)–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2 derived from the corresponding Z(OMe)-derivative[6] by the TFA treatment.[14] The DCC plus N-hydroxybenzotriazole (HOBT) procedure[15] was employed for this condensation reaction to minimize the acylurea formation known as a side reaction of DCC. The resulting protected dodecapeptide amide, Z(OMe)–Tyr–Arg(NO_2)–Pro–Lys(Z)–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2, was purified by column chromatography on silica using the solvent system of chloroform, methanol and water (8: 3: 1) and its purity was confirmed by three criteria: thin-layer chromatography, elemental analysis and hydrolysis with 3N *p*-toluenesulfonic acid.[16]

Removal of all protecting group from the above protected peptide amide was performed by hydrogen fluoride[17] in the presence of anisole containing 2% ethanedithiol in an ice-bath for 45 minutes. The deblocked peptide was soon converted to the corresponding acetate by treatment with Amberlite IR-4B and then incubated with dithiothreitol to reduce Met sulfoxide formed partially during the above treatment. The product was subsequently purified by column chromatography on Sephadex G-10 using 30% acetic acid as eluent. The product obtained at this purification stage exhibited a single spot on thin-layer chromatography in two different solvent systems. Its purity was further confirmed by elemental analysis and hydrolysis with 3N *p*-toluenesulfonic acid. When contractility on isolated guinea-pig ilia was examined, synthetic Tyr^1-substance P exhibited a half of the activity of our synthetic substance P.

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Experimental

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems. Rf^1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf^2 *n*–BuOH–pyridine–AcOH–H_2O (30:20:6:24), Rf^3 *n*–BuOH–pyridine–AcOH–H_2O (4:1:1:2).

Z(OMe)-Tyr-Arg(NO_2)-Pro-OH——Z-Arg(NO_2)-Pro-OH (4.50 g) was treated with 30% HBr–AcOH (20 ml) in an ice-bath for 45 min and dry ether was added. The resulting white powder was collected by filtration, dried over KOH pellets *in vacuo* for 5 hr and then dissolved in DMF (30 ml). Et_3N (1.4 ml) was added and the solution was kept in an ice-bath until the following azide was prepared. To a solution of Z(OMe)-Tyr-$NHNH_2$ (3.60 g) in DMF (70 ml), 3.13 N HCl–DMF (6.4 ml) and isoamylnitrite (1.33 ml) were added under cooling with ice–NaCl and the solution was stirred for 5 min, until the hydrazine test[18] became nagative. The solution, after neutralization with Et_3N (2.8 ml), was combined with a solution of H–Arg-(NO_2)–Pro–OH prepared above. The mixture was stirred at 4° for 48 hr. A few drops of AcOH was added and the solvent was evaporated *in vacuo*. The residue was dissolved in 5% NH_4OH which, after washing with ethyl acetate, was acidified with citric acid. The resulting precipitate was extracted with ethyl acetate, which was washed with H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and then precipitated from ethyl acetate with ether; yield 3.53 g (55%), mp 124—128°, $[\alpha]_D^{26}$ −46.2° (c=0.5, MeOH). Rf^1 0.11. *Anal.* Calcd. for $C_{29}H_{37}O_{10}N_7 \cdot H_2O$: C, 52.63; H, 5.94; N, 14.81. Found: C, 53.05; H, 6.29; N, 14.52.

Z(OMe)-Tyr-Arg(NO_2)-Pro-Lys(Z)-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2——Z(OMe)-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (1.39 g) was treated with TFA (3.0 ml) in the presence of anisole (0.7 ml) in an ice-bath for 45 min and then dry ether was added. The resulting powder was collected by filtration, dissolved in 1N HCl–DMF (0.99 ml) and again dry ether was added. The resulting hydrochloride, isolated as a fine powder, was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (20 ml). To this solution, Et_3N (0.14 ml). Z(OMe)-Tyr-Arg(NO_2)-Pro-OH (0.80 g), HOBT (0.16 g) and DCC (0.25 g) were successively added and the mixture was stirred at room temperature for 48 hr. The solution, after filtration, was condensed *in vacuo* and the residue was treated with ethyl acetate. The resulting powder was washed batchwisely with 10% citric acid, 5% Na_2CO_3 and H_2O and then dissolved in a small amount of the solvent consisting of $CHCl_3$–MeOH–H_2O (8:3:1). The solution was applied to a column of silica (2.8×15 cm), which was eluted with the same solvent system. Fractions containing the substance of Rf^1 0.56 were combined and the solvent was evaporated. The residue was treated with H_2O and the resulting powder was precipitated from DMF with ethyl acetate; yield 0.69 g (37%), mp 210—213°, $[\alpha]_D^{27}$ −45.3° (c=0.5, DMF). Rf^1 0.56. Amino acid ratios in a Tos-OH hydrolysate: Tyr 0.67, Arg not determined, Pro 1.89, Lys 1.04, Glu 1.99, Phe 1.97, Gly 1.00, Leu 0.84, Met 0.86 (average recovery 86%). *Anal.* Calcd. for $C_{89}H_{120}O_{22}N_{20}S \cdot 2H_2O$: C, 56.55; H, 6.61; N, 14.82. Found: C, 56.40; H, 6.38; N, 14.43.

H-Tyr-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2——The above protected dodecapeptide amide (130 mg) was treated with HF (approximately 5 ml) in the presence of anisole containing 2% ethanedithiol (1.0 ml) in an ice-bath for 40 min. The excess HF was removed by evaporation and the residue was dissolved in ice-cooled H_2O (5 ml), which was treated with Amberlite IR-4B (acetate form, approximately 3 g) for 30 min. The resin was removed by filtration and the filtrate, after washing with ethyl acetate, was lyophilized. The resulting powder was dissolved in 30% AcOH (6 ml) and dithiothreitol (20 mg) was added. The solution was then incubated at 50° for 8 hr and applied to a column of Sephadex G-10 (2.8×96 cm), which was eluted with 30% AcOH. Individual fractions (4 ml each) were collected and absorbancy at 275 mμ was determined. Fractions (tube No. 37—53) were combined, the solvent was evaporated *in vacuo* and the residue was lyophilized to give a fluffy white powder; yield 96 mg (75%), $[\alpha]_D^{27}$ −73.5° (c=0.4, 10% AcOH), Rf^2 0.60, Rf^3 0.45. Amino acid ratios in a Tos-OH hydrolysate: Tyr 0.74, Arg 0.86, Pro 1.99, Lys 0.94, Glu 2.08, Phe 2.02, Gly 1.00, Leu 0.87, Met 0.75 (average recovery 81%). Amino acid ratios in AP-M digest:[19] Tyr 0.85, Arg 0.83, Pro 1.75, Lys 1.06, Gln 1.75, Phe 2.27, Gly 1.00, Leu 1.04, Met 0.79 (average recovery 78%). *Anal.* Calcd. for $C_{78}H_{119}O_{21}N_{19}S \cdot 3CH_3COOH \cdot 8H_2O$: C, 51.04; H, 7.41; N, 14.50. Found: C, 50.83; H, 7.22; N, 14.77.

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