to be 350 times sweeter than sucrose, and its sweet level was comparable to that of phyllodulcin.

From these results, it may be concluded that the relationship between sweet taste and structure of 5-hydroxyflavanones lacking 7-glycoside moiety is similar to that of 3,4-dihydroisocoumarins.

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Synthesis of the Nonatetracontapeptide corresponding to the Sequence proposed for Thymopoietin II[1)]

First total synthesis of a nonatetracontapeptide corresponding to the entire amino acid sequence of thymopoietin II, a T cell differentiating hormone isolated from bovine thymus, was described. Toward the synthesis of this hormone, two relatively large fragments corresponding to the sequence 1—27 and 28—49 were prepared and the fragments were served as the building blocks for the final construction of the entire amino acid sequence of the hormone. The final deprotection of the fully protected nonatetracontapeptide was achieved by the treatment with methanesulfonic acid or hydrogen fluoride, and the purification of the synthetic peptide was effected by a column chromatography on Sephadex G-50, Biogel P-2 and CM-cellulose. The physicochemical and biological properties of the synthetic peptide were also described.

Keywords——thymopoietin II; thymocyte differentiation; nonatetracontapeptide; fragment condensation; methanesulfonic acid

The entire amino acid sequence of bovine thymopoietin II (Tp), a T cell differentiating hormone of the thymus, was recently elucidated by Schlesinger and Goldstein.[2)] We have synthesized the nonatetracontapeptide corresponding to the entire sequence of this unique hormone. To date only partial synthesis of Tp, tridecapeptide (29—41), by the solid phase method has been described.[3)]

In our present synthesis of Tp in solution (Fig. 1), amino acid derivatives bearing protecting groups, *e.g.*, Z, OBzl and MBS, finally removable by the treatment with methanesulfonic acid (MSA)[4)] were employed. Among these protecting groups, the MBS group was recently

1) Amino acids, peptides and their derivatives in this communication are the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, BOC=*tert*-butoxycarbonyl, MBS=*p*-methoxybenzenesulfonyl, OBzl=benzyl ester, OBu^t=*tert*-butyl ester, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, DCC=N,N′-dicyclohexylcarbodiimide, DMF=N,N′-dimethylformamide, TEA=triethylamine, TFA=trifluoroacetic acid. Solvent systems for thin-layer (silica gel, Merck 60 F-254 plate) chromatography are: Rf^1=$CHCl_3$–MeOH–AcOH (9:1:0.5), Rf^2=$CHCl_3$–MeOH–AcOH (8:2:0.5), Rf^3=$CHCl_3$–MeOH–AcOH (8:3:1), Rf^4=AcOEt–pyridine–AcOH–H_2O (60:20:6:10), Rf^5=*n*-BuOH–AcOH–H_2O (4:1:1), Rf^6=*n*-BuOH–AcOEt–AcOH–H_2O (1:1:1:1), Rf^7=*n*-BuOH–pyridine–AcOH–H_2O (30:20:6:24).

2) D.H Schlesinger and G. Goldstein, *Cell*, **5**, 361 (1975).
3) D.H. Schlesinger and G. Goldstein, M.P. Scheid and E.A. Boyse, *Cell*, **5**, 367 (1975).
4) H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).

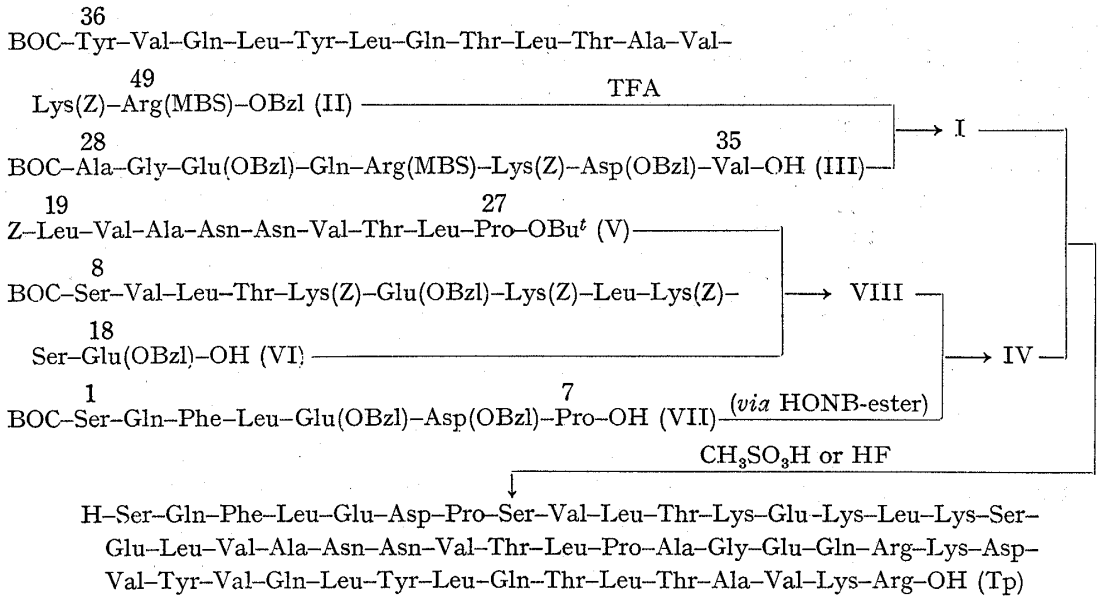

Fig. 1. Synthetic Route to Thymopoietin II

reported from this laboratory[5] to be useful for the protection of the guanidino function of arginine. The α-amino function of intermediates was protected by the BOC-group. Most of the couplings of the intermediates were achieved by the HONB-DCC procedure[6] to minimize the undesirable epimerization during the peptide-bond forming reactions. The intermediates were mainly built up by the stepwise elongation manner and purified by reprecipitation from a suitable solvent.

Toward the synthesis of Tp, two relatively large protected fragments, 1—27 and 28—49, were prepared as the building blocks.

For the synthesis of the C-terminal docosapeptide corresponding to the sequence 28—49, BOC–Ala–Gly–Glu(OBzl)–Gln–Arg(MBS)–Lys(Z)–Asp(OBzl)–Val–Tyr–Val–Gln–Leu–Tyr–Leu–Gln–Thr–Leu–Thr–Ala–Val–Lys(Z)–Arg(MBS)–OBzl (I) [mp 275—280° (decomp.); $[\alpha]_D^{26}$ +2.7° (c=0.4, DMF); Rf^2 0.69; *Anal.* Calcd. for $C_{171}H_{241}O_{46}N_{33}S_2 \cdot 6H_2O$: C, 56.00; H, 6.95; N, 12.60; S, 1.75. Found: C, 55.69; H, 6.70; N, 12.44; S, 1.67.; amino acid analysis (acid hydrolysis)[7]: Arg 2.00, Lys 2.10, Asp 0.90, Thr 2.16, Glu 3.70, Gly 1.00, Ala 2.00, Val 2.60, Leu 2.74, Tyr 0.58[8]], two peptide fragments, protected tetradecapeptide (II) [mp 260—262° (decomp.); $[\alpha]_D^{26}$ −11.0° (c=0.4, DMF); Rf^2 0.60; *Anal.* Calcd. for $C_{106}H_{156}O_{28}N_{20}S \cdot H_2O$: C, 57.64; H, 7.21; N, 12.68; S, 1.45. Found: C, 57.11; H, 7.23; N, 12.42; S, 1.42.] and octapeptide (III) [mp 203—205° (decomp.); $[\alpha]_D^{26}$ −22.6° (c=0.5, DMF); Rf^2 0.50; *Anal.* Calcd. for $C_{70}H_{95}O_{21}N_{13}S \cdot 2H_2O$: C, 55.21; H, 6.55; N, 11.96; S, 2.10. Found: C, 55.07; H, 6.34; N, 11.94; S, 2.00], were prepared. The protected peptide II was treated with TFA and the resulting partially deblocked peptide was converted to the corresponding free base by the treatment with TEA. The resulting free base was then condensed with the protected peptide III by the HONB-DCC method to give the protected docosapeptide I.

The N-terminal heptacosapeptide corresponding to the sequence 1—27, BOC–Ser–Gln–Phe–Leu–Glu(OBzl)–Asp(OBzl)–Pro–Ser–Val–Leu–Thr–Lys(Z)–Glu(OBzl)–Lys(Z)–Leu–Lyz–(Z)–Ser–Glu(OBzl)–Leu–Val–Ala–Asn–Asn–Val–Thr–Leu–Pro–OH (IV) [mp 274° (decomp.); $[\alpha]_D^{23}$ −18.4° (c=0.5. DMF); Rf^3 0.12; Rf^4 0.75; Rf^7 0.76; *Anal.* Calcd. for $C_{195}H_{281}O_{52}N_{33} \cdot$

5) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **24**, 1568 (1976).
6) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1857 (1974).
7) Acid hydrolysis: 5.7N HCl, 105°, 24 hr.
8) Partial alkylation should be occurred during hydrolysis.

$2H_2O$: C, 59.20; H, 7.26; N, 11.69. Found: C, 59.05; H, 7.18; N, 11.93.; amino acid analysis (acid hydrolysis)[7]: Lys 2.92, Asp 2.80, Thr 1.92, Ser 2.70, Glu 3.91, Pro 1.78, Ala 1.00, Val 3.00, Leu 4.70, Phe 0.87] was prepared by the two step fragment condensation from V [mp 258° (decomp.); $[\alpha]_D^{22}$ −41.8° (c=0.4, DMF); Rf^5 0.70; Rf^6 0.89; *Anal.* Calcd. for $C_{54}H_{87}O_{15}N_{11}\cdot H_2O$: C, 56.48; H, 7.81; N, 13.42. Found: C, 56.51; H, 7.84; N, 13.64], VI [mp 246° (decomp.); $[\alpha]_D^{22}$ −9.8° (c=0.5, DMF); Rf^1 0.18; Rf^4 0.59; *Anal.* Calcd. for $C_{98}H_{138}O_{27}N_{14}\cdot H_2O$: C, 59.98; H, 7.19; N, 9.99. Found: C, 59.93; H, 7.24; N, 10.02] and VII [mp 204° (decomp.); $[\alpha]_D^{22}$ −36.0° (c=0.5, DMF); Rf^1 0.15; *Anal.* Calcd. for $C_{50}H_{74}O_{16}N_8$: C, 60.31; H, 6.69; N, 10.05. Found: C, 60.05; H, 6.69; N, 9.91]. The protected peptide V was hydrogenated to remove the N^{α}–Z-group and the resulting free base was acylated with VI by the HONB-DCC method to give the fully protected eicosapeptide, BOC–Ser–Val–Leu–Thr–Lys(Z)–Glu(OBzl)–Lys(Z)–Leu–Lys(Z)–Ser–Glu(OBzl)–Leu–Val–Ala–Asn–Asn–Val–Thr–Leu–Pro–OBu^t (VIII) [mp 283° (decomp.); $[\alpha]_D^{22}$ −17.5° (c=0.5, DMF); Rf^1 0.22; Rf^3 0.46; *Anal.* Calcd. for $C_{144}H_{217}O_{79}N_{25}\cdot H_2O$: C, 58.82; H, 7.57; N, 11.91. Found: C, 58.77; H, 7.47; N, 11.83]. The N^{α}–BOC-group and the C-terminal OBu^t of this protected peptide VIII were removed by the TFA-treatment, and the resulting partially protected peptide was acylated with the HONB ester of VII to give the protected peptide IV.

The relatively large intermediates were then condensed by the HONB–DCC method in N-methyl-2-pyrrolidone as a solvent, because of their poor solubility in DMF. The resulting crude protected peptide was precipitated by the addition of an aqueous sodium bicarbonate solution and the precipitate was collected by filtration and washed well with acetonitrile and ethylacetate. The crude peptide thus obtained, without further purification, was deblocked with MSA in the presence of anisole at 18° for 100 min or with anhydrous hydrogen fluoride[9] in the presence of anisole at 0° for 50 min to give the crude Tp. The crude Tp was immediately converted to the corresponding acetate with Amberlite CG-400 (acetate form) and purified by gel filtration on Sephadex G-50 and Biogel P-2, followed by further purification with CM-cellulose by gradient elution using pH 6.5 ammonium acetate buffer (0.001→0.3M). Absorbency at 206 nm due to the peptide-bond served to monitor the chromatographic purifications.

The nonatetracontapeptide thus obtained exhibited a single spot (ninhydrin and Sakaguchi reagents) on thin-layer chromatography in two different solvent systems [Rf^6 0.60; Rf^6 (cellulose) 0.68; Rf^7 (cellulose) 0.59] and on paper electrophoresis [pH 1.9 formic acid–acetic acid

Table I. Induction of Thymocyte Antigen (Thy-1) on Prothymocytes by Synthetic Peptides[a]

Peptide concentration (μg/ml)	Induction index (%)[b]	
	Tridecapeptide (29—41)	Nonatetracontapeptide (1—49)
0.001	4.67±1.60[c]	9.50±0.97[c]
0.01	7.87±1.79	16.10±1.46
0.1	12.50±1.13	16.33±2.30
1.0	13.37±2.90	15.80±0.85
10.0	15.00±2.51	14.10±1.37

a) Prothymocytes were obtained from C3H mouse spleen cells of the B layer fractionated by the discontinuous bovine serum albumin density gradient centrifugation.[10]
b) Induction index was calculated according to the formula $100\times A-B/B$, where A is percentage of Thy-1 negative cells cultured without peptide, and B is percentage of Thy-1 negative cells cultured with peptide.
c) Figures represent the mean value of triplicate culture and the standard error of the mean.

9) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **87**, 4922 (1965).
10) K. Komuro and E. Boyse, *Lancent*, **1**, 740 (1973).

buffer, 600 volt, 60 min: 0.59×Lys]: $[\alpha]_D^{25}$ −75° (c=0.3, 5% aqueous AcOH); amino acid analysis (acid hydrolysis)[7,11]: Arg 1.98, Lys 5.20, Asp 4.11, Thr 4.00, Ser 3.16, Glu 8.16, Pro 2.02, Gly 1.00, Ala 3.04, Val 6.00, Leu 8.06, Tyr 1.36, Phe 1.08 (recovery, 76%).

The synthetic peptide was active in the induction of thymocyte differentiation from prothymocyte *in vitro*[10] (Table I) and the potency of the synthetic hormone was more than 10 times the potency of the synthetic tridecapeptide corresponding to the sequence 29—41 of the hormone.[12] Since it has been fully confirmed by Schlesinger, *et al.*[3] that the fragment 29—41 exhibited approximately 10% activity by weight when compared with natural Tp, the activity of our synthetic hormone would consequently be comparable to that of natural Tp. Further biological characterization of this synthetic hormone is now in progress in these laboratories.[13]

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11) After enzymatic hydrolysis by chymotrypsin and aminopeptidase-M, the amino acid composition of the product was also good agreement with the theory.
12) The tridecapeptide (29—41) was prepared by the conventional stepwise elongation method: $[\alpha]_D^{26}$ −34.0° (c=0.1 in 50% AcOH); Rf^7 (cellulose) 0.54.
13) The detailed biological studies will be reported elsewhere by Drs. Kawaji, Takaoki and Sugino.

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Oxidation of 4'-Substituted Benzenesulfenanilides with Lead Dioxide. Electron Spin Resonance Studies of Benzenesulfenanilidyl Radicals

Benzenesulfenanilidyl radicals (3) were generated by the oxidation of benzenesulfenanilides (4'-OMe (**1a**), 4'-Me (**1b**), 4'-Cl (**1c**), 4'-H (**1d**)) with lead dioxide, and their electron spin resonance (ESR) and visible spectra were investigated. The radicals generated from **1a** and **1b** in benzene were fairly stable and gave well-resolved ESR spectra, whereas those from **1c** and **1d** were less stable and gave poorly resolved ESR spectra. The oxidations of **1a** and **1b** gave the corresponding phenazines as the final products, whereas those of **1c** and **1d** did not.

Keywords——benzenesulfenanilides; oxidation of sulfenanilides; oxidation with lead dioxide; electron spin resonance; benzenesulfenamidyl radicals; nitrene; imido intermediate; synthesis of phenazines

In the previous paper[1] we reported the results on the controlled potential electrolyses of benzenesulfenanilides (4'-OMe (**1a**), 4'-Me (**1b**), 4'-Cl (**1c**), 4'-H (**1d**)) and 2-nitrobenzenesulfenanilides (4'-OMe (**2a**), 4'-Me (**2b**), 4'-Cl (**2c**), 4'-H (**2d**)) in acetonitrile. Electrolyses of **1a**, **1b**, **2a**, **2b**, and **2c** gave the corresponding 2,7-disubstituted phenazines, whereas those of **1c**,

1) H. Sayo, K. Mori, and A. Ueda, *Chem. Pharm. Bull.* (Tokyo), **25**, 525 (1977).