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Synthesis and Central Nervous System Actions of Thyrotropin-Releasing Hormone Analogs Containing a 1-Oxo-1,2,3,4-tetrahydroisoquinoline Moiety[1)]

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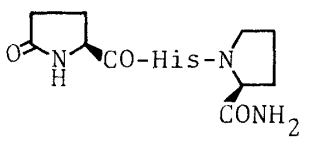
In order to find compounds having selective central nervous system (CNS) actions, various thyrotropin-releasing hormone (TRH) analogs in which the pyroglutamic acid residue is replaced by (3*S*)-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Otc–OH) and related derivatives were prepared and their CNS actions were investigated in mice.

Otc–His–Pro–NH_2 (**9a**) showed 3.5—10 times stronger CNS actions than TRH (**1**). However, it was also 3—4 times more potent than TRH in thyrotropin (TSH)-releasing activity.

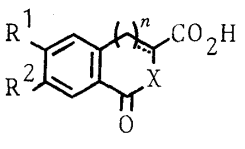
Keywords——TRH analog; central nervous system; 1-oxo-1,2,3,4-tetrahydroisoquinoline; spontaneous locomotor activity; reserpine-induced hypothermia effect; pentobarbital anesthesia effect

Recently much attention has been paid to the direct central nervous system (CNS) actions[2)] of thyrotropin releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-proline amide, **1**). Since TRH itself has thyrotropin (TSH)-releasing activity from the anterior pituitary,[3)] separation of the CNS actions and the endocrine action is an important target for developing efficient drugs. From this point of view, several chemical modifications of the parent molecule **1** have been reported.[4–8)] Among them, DN-1417[4)] in which pyrrolidone in position 1 of TRH was replaced by γ-butyrolactone and MK-771[5)] in which both the pyrrolidone group and the C-terminal proline amide moiety of TRH were replaced by piperidone group and thiazolidine carboxamide, respectively, have been evaluated as potent CNS activators.

In this context, we also decided to carry out chemical modifications to gain some information about the biological effects of lipophilic residues on the CNS. We synthesized TRH analogs of a new type, containing (3*S*)-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Otc–OH, **2a**), and related compounds (**2b—q**), which have lipophilic residues in place of the pyroglutamic acid residue in position 1 of TRH (**1**). The CNS actions of these TRH analogs **9a—q** and **13** were compared with those of TRH. Moreover, the TSH-releasing activity of the analog **9a** showing the most potent CNS actions was measured.



TRH (**1**)



2a—q

Chart 1

Chemistry

1-Oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (Oct–OH) Derivatives——(3*S*)- and (3*R*)-Otc–OH (**2a** and **2b**) were synthesized *via* oxidation and recyclization of the isoquinoline carboxylic acids **4a** and **4b**, using L-Phe (**3a**) and D-Phe (**3b**) as starting materials, according to the known methods.[9] In a similar way, the corresponding 6,7-dihydroxy- and 6,7-dimethoxy-substituted isoquinoline compounds **2c** and **2d** were derived from 3,4-dihydroxyphenylalanine (DOPA) (**3c**) as shown in Chart 2.

First, the tetrahydroisoquinoline carboxylic acid **4c**[10] prepared from **3c** and formaldehyde was transformed to the 6,7-dimethoxy compound **5d** by treatment with benzoyl chloride followed by dimethylation. Then, **5d** was oxidized to give the dicarboxylic acid **6d**, which was converted to the desired product **2d** by cyclization with 6 N HCl. Furthermore, the 6,7-dihydroxy carboxylic acid **2c** was obtained by treatment with a mixture of aqueous HBr and acetic acid followed by saponification.

Several 7-substituted Otc–OH derivatives **2e—i** were prepared *via* the nitration[11] of the

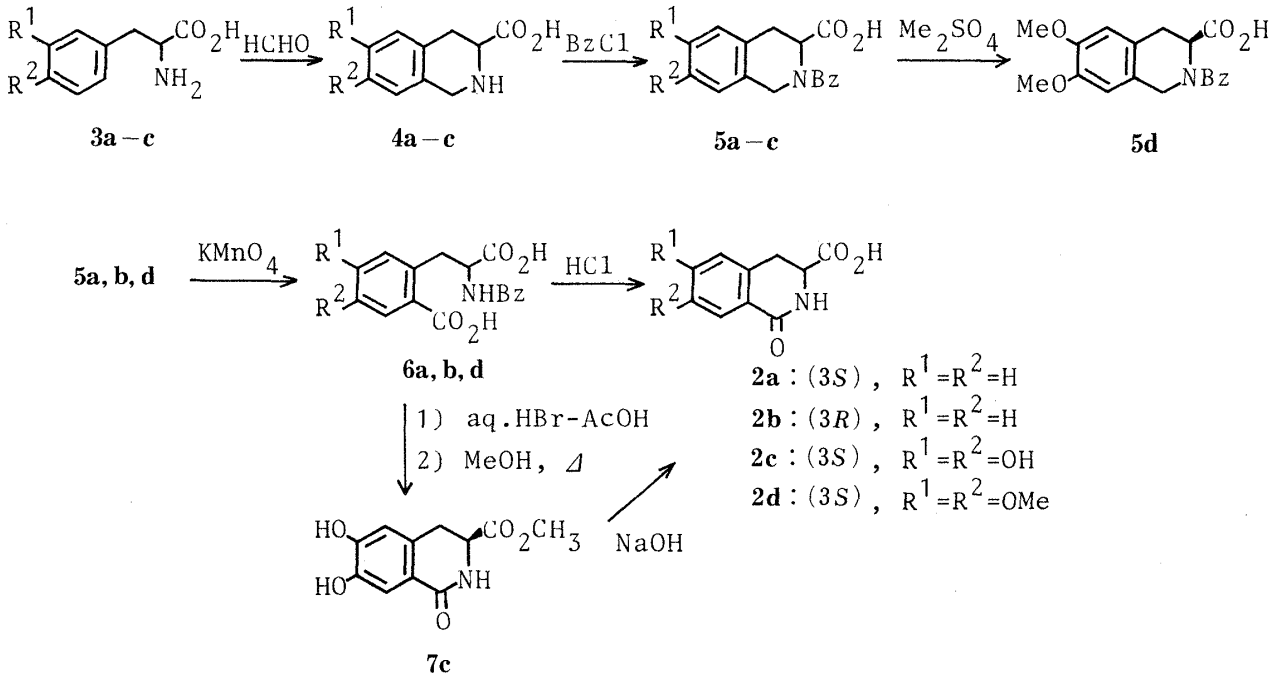


Chart 2

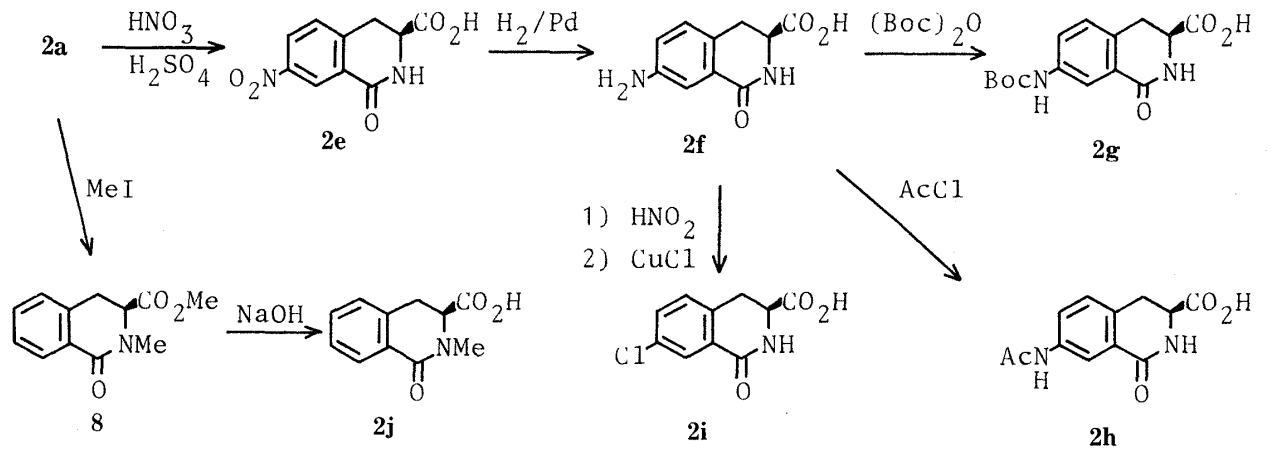
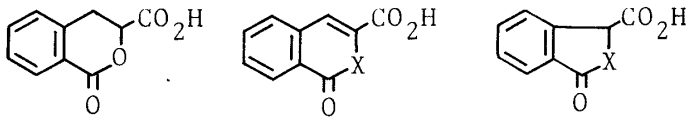


Chart 3

original carboxylic acid Otc–OH (**2a**). The nitro group of **2e** was reduced to the amino group by catalytic hydrogenation to afford **2f**, which was converted to the acylated amino acids **2g** and **2h**. The 7-chloro-substituted carboxylic acid **2i** was prepared by a usual Sandmeyer reaction of the amino acid **2f**. On the other hand, alkylation of the nitrogen atom at the 2-position of **2a** was also carried out. Namely, the 2-methyl derivative **2j** was prepared by using methyl iodide and silver oxide followed by hydrolysis of the methyl ester **8**. These procedures are shown in Chart 3.

Moreover, the following related compounds were also prepared as Otc–OH analogs (Chart 4). Among them, (±)-3,4-dihydroisocoumarin-3-carboxylic acid (**2k**),[12] isocarbostyril-3-carboxylic acid (**2n**),[13] isocoumarin-3-carboxylic acid (**2o**),[14] (±)-phthalimidine-3-carboxylic acid (**2p**),[15] and (±)-phthalide-3-carboxylic acid (**2q**)[16] were prepared by the reported methods.

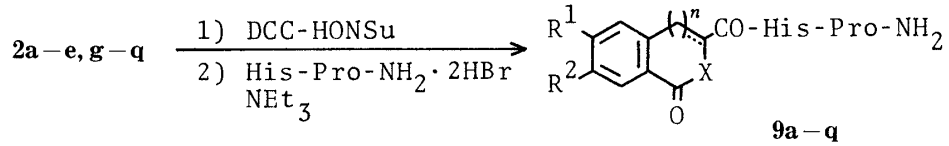
For the preparation of (3*S*)- and (3*R*)-3,4-dihydroisocoumarin-3-carboxylic acids (**2l** and **2m**), although the hydrolytic resolution of methyl 3,4-dihydroisocoumarin-3-carboxylate with α-chymotrypsin and a stereospecific synthesis from optically active phenyllactic acid have been reported,[17] we carried out the direct optical resolution of the racemic acid **2k** using chiral Phe–NH_2. Consequently, the (3*S*)-isomer (**2l**) was obtained from D-Phe–NH_2 and the (3*R*)-isomer (**2m**) from L-Phe–NH_2; they showed higher optical rotations than the reported values, as described in the experimental section.



2k : (±) **2n** : X=NH **2p** : (±), X=NH
2l : (3*S*) **2o** : X=O **2q** : (±), X=O
2m : (3*R*)

Chart 4

Synthesis of TRH Analogs——Conversions of the Otc–OH-related compounds obtained to the TRH analogs were carried out by a conventional DCC coupling method. Namely, the carboxylic acids **2a—e** and **2g—q** were condensed with the dipeptide amide His–Pro–NH_2, which has been used for TRH analog synthesis,[18] in the presence of HONSu as shown in Chart 5.



9a—q

Chart 5

The coupling products were separated on a column packed with MCI GEL CHP-20P by passing aqueous MeOH as an eluent. This procedure was particularly favorable for the removal of an undesired side product, cyclo(His–Pro).[19] The purified TRH analogs were isolated as crystals or amorphous substances by lyophilization. On the other hand, derivatives insoluble in water were treated with diluted HCl and identified as their soluble hydrochlorides **9e**, **9n**, and **9o**. The tripeptide **9f** having the amino group at the 7-position on tetrahydroisoquinoline was prepared by acidolytic removal of the Boc protecting group of **9g**.

Furthermore, Pro–NH_2 (the C-terminal residue) of **9a** was replaced by L-thiazolidine-4-carboxamide (Ṫzl–NH_2),[5b] to obtain a TRH analog which was expected to show more

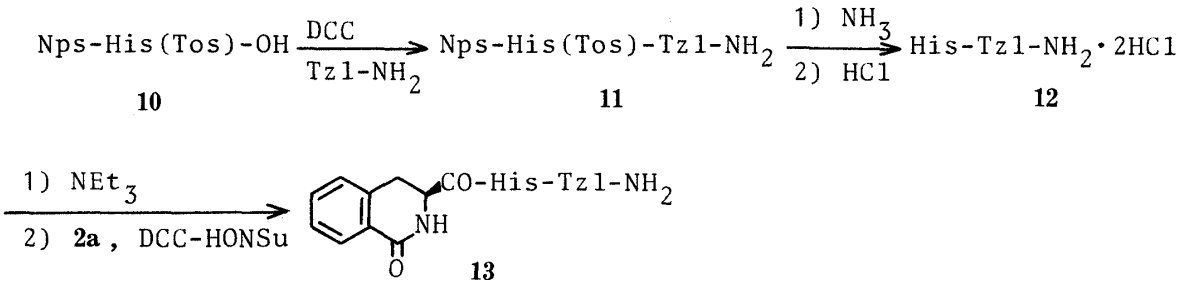


Chart 6

TABLE I. Physical Constants and Analytical Data of TRH Analogs

No.	TLC[a]			mp (dec.) (°C) Recrystn. solvent	$[\alpha]_D^{25}$ (deg.) ($c=1$, H_2O)	Yield (%)	Formula	Analysis (%) Calcd (Found)		
	Rf_1	Rf_2	Rf_3					C	H	N
9a	0.54	0.29	0.35	101—103 H_2O	+25.6	50	$C_{21}H_{24}N_6O_4 \cdot 5/4\,H_2O$	56.42 (56.32)	5.97 (5.76)	18.80 (18.75)
9b	0.54	0.29	0.35	A[b]	−131.0	42	$C_{21}H_{24}N_6O_4 \cdot H_2O$	57.00 (56.70)	5.92 (5.72)	18.99 (19.28)
9c	0.42	0.22	0.33	199—204 H_2O	−10.0[c]	37	$C_{21}H_{24}N_6O_6 \cdot 3/2\,H_2O$	52.17 (52.04)	5.63 (5.40)	17.39 (17.49)
9d	0.43	0.20	0.36	A	+19.2	63	$C_{23}H_{28}N_6O_6 \cdot 5/3\,H_2O$	53.68 (53.42)	6.14 (5.63)	16.34 (16.04)
9e	0.62	0.25	0.45	A	+21.6	70	$C_{21}H_{37}N_7O_6 \cdot HCl \cdot 3H_2O$	45.04 (44.75)	5.22 (5.46)	17.51 (17.13)
9f	0.24	0.10	0.26	A	−24.5	100	$C_{21}H_{25}N_7O_4 \cdot H_2O$	55.13 (54.99)	5.94 (5.91)	21.43 (21.55)
9g	0.68	0.41	0.48	A	−12.8	33	$C_{26}H_{33}N_7O_6 \cdot 2H_2O$	54.25 (54.32)	6.47 (6.14)	17.03 (17.49)
9h	0.35	0.17	0.31	A	−3.6	64	$C_{23}H_{27}N_7O_5 \cdot 7/4\,H_2O$	53.84 (53.82)	5.99 (5.80)	19.12 (19.25)
9i	0.62	0.35	0.44	A	+5.4	67	$C_{21}H_{23}ClN_6O_4 \cdot H_2O$	52.88 (52.74)	5.28 (5.06)	17.63 (17.73)
9j	0.52	0.23	0.39	A	−50.2	48	$C_{22}H_{26}N_6O_4 \cdot H_2O$	57.88 (58.25)	6.18 (5.92)	18.41 (18.40)
9k	0.63	0.34	0.48	A	−65.8	47	$C_{21}H_{23}N_5O_5 \cdot H_2O$	56.87 (57.13)	5.68 (5.43)	15.80 (15.76)
9l	0.63	0.34	0.48	A	−60.7	56	$C_{21}H_{23}N_5O_5$	59.28 (59.38)	5.45 (5.56)	16.47 (16.39)
9m	0.63	0.34	0.48	A	−72.9	52	$C_{21}H_{23}N_5O_5$	59.28 (59.50)	5.45 (5.30)	16.47 (16.45)
9n	0.63	0.33	0.40	A	−96.2	63	$C_{21}H_{22}N_6O_4 \cdot HCl \cdot 4/3\,H_2O$	52.22 (52.12)	5.36 (5.16)	17.41 (17.36)
9o	0.64	0.37	0.48	A	−81.2	31	$C_{21}H_{21}N_5O_5 \cdot HCl \cdot 7/4\,H_2O$	51.32 (51.34)	5.23 (4.90)	14.25 (14.12)
9p	0.52	0.24	0.35	A	−68.6	34	$C_{20}H_{22}N_6O_4 \cdot 5/2\,H_2O$	52.74 (52.64)	5.98 (5.21)	18.46 (18.36)
9q	0.59	0.34	0.35	220—223 H_2O	−34.4[c]	43	$C_{20}H_{21}N_5O_5$	58.38 (58.27)	5.15 (5.08)	17.03 (16.95)
13	0.64	0.38	0.32	A	−6.4	42	$C_{20}H_{22}N_6O_4S \cdot 5/4\,H_2O$	51.65 (51.63)	5.31 (5.04)	18.07 (17.58)

a) See experimental section for details. *b*) A = amorphous powder. *c*) In MeOH.

powerful CNS actions. As shown in Chart 6, Nps–His(Tos)–OH (**10**), which was synthesized by the known procedure,[20a] was coupled with Tzl–NH_2 in the presence of DCC to give **11**. The tosyl group was deblocked by NH_3 in MeOH[20b] followed by removal of the Nps group by acidolysis[21] to afford the salt of the dipeptide amide, His–Tzl–NH_2·2HCl (**12**). The coupling reaction between the carboxylic acid **2a** and the dipeptide amide **12** was performed to obtain the desired product **13**.

The identification of the products **9a**—**q** and **13** was confirmed by elemental analysis, thin layer chromatography (TLC), paper electrophoresis, and/or high-pressure liquid chromatography (HPLC). Physical data and yields of these TRH analogs are summarized in Table I.

Biological Results and Discussion

The CNS actions of the resulting TRH analogs **9a**—**q** and **13** containing 1-oxo-1,2,3,4-

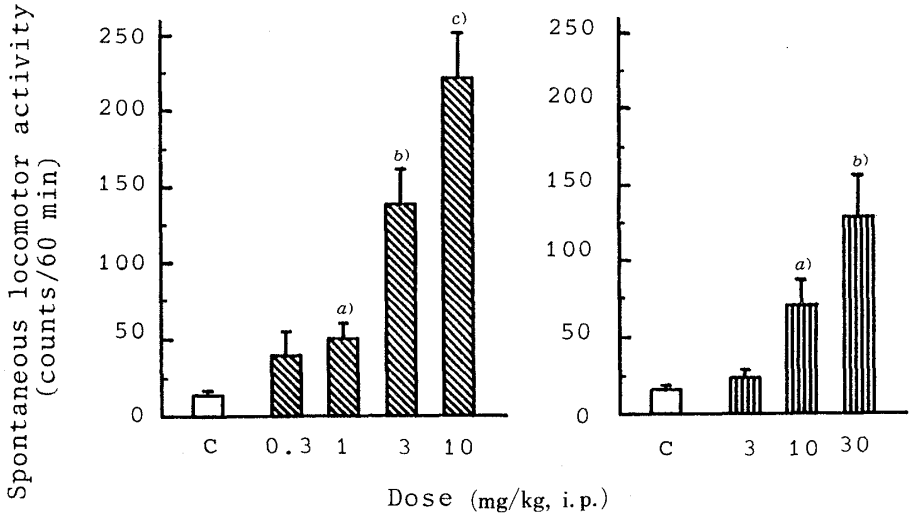


Fig. 1. Effects of **9a** (Left) and TRH (Right) on Spontaneous Locomotor Activity in Mice

Each bar represents the mean ± standard error of 4—5 mice. Significant differences are indicated as *a*) $p<0.05$, *b*) $p<0.01$, and *c*) $p<0.001$ *vs.* control value. C, control (saline).

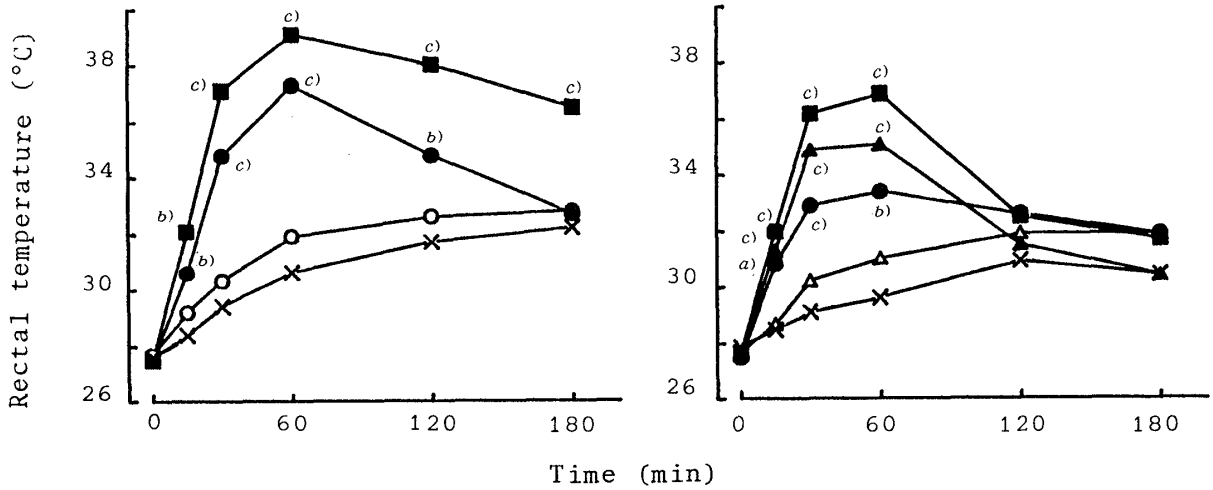


Fig. 2. Effects of **9a** (Left) and TRH (Right) on Reserpine-Induced Hypothermia in Mice

Each point represents the mean value of 5 mice. Significant differences are indicated as *a*) $p<0.05$, *b*) $p<0.01$, and *c*) $p<0.001$ *vs.* control value. ×, control; ○, 0.1 mg/kg, i.p.; △, 0.3 mg/kg, i.p.; ●, 1 mg/kg, i.p.; ▲, 3 mg/kg, i.p.; ■, 10 mg/kg, i.p.

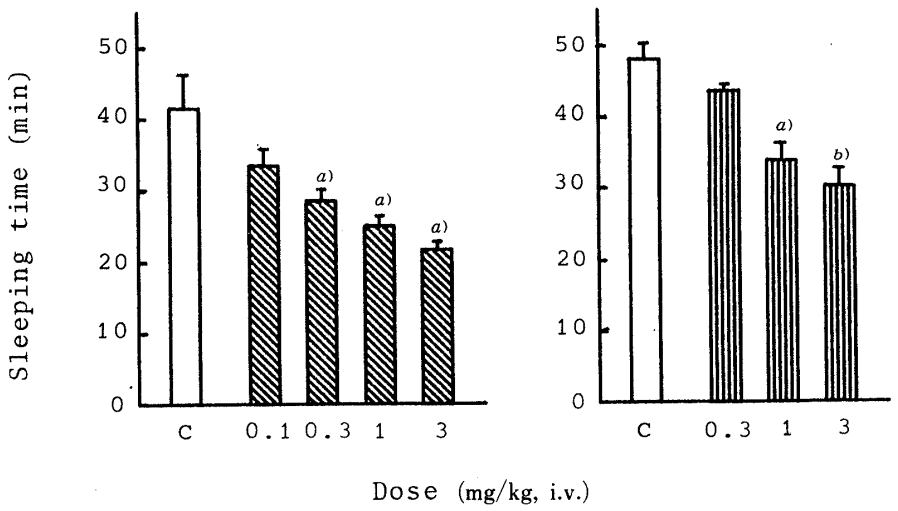


Fig. 3. Effects of **9a** (Left) and TRH (Right) on Pentobarbital Anesthesia in Mice

Each bar represents the mean ± standard error of 6 mice. Significant differences are indicated as *a*) $p<0.01$ and *b*) $p<0.001$ *vs.* control value. C, control (saline).

TABLE II. Central Nervous System Actions of TRH Analogs[a)]

No.	L[b)] (i.p.)	R[c)] (i.p.)	P[d)] (i.v.)	No.	L[b)] (i.p.)	R[c)] (i.p.)	P[d)] (i.v.)
9a	11.2	10.1	3.5	**9k**	2.8	0.8	1.4
9b	4.9	5.1	2.2	**9l**	6.0	4.8	1.5
9c	3.9	8.4	1.0	**9m**	—	0.5	0.7
9d	—[e)]	1.1	1.0	**9n**	3.3	1.5	0.9
9e	4.4	3.2	0.7	**9o**	—	0.4	—
9f	6.2	4.4	1.3	**9p**	0.6	0.1	0.6
9g	—	0.1	0.3	**9q**	0.3	0.1	—
9h	—	0.4	0.3	**13**	1.6	5.6	1.3
9i	3.8	3.7	3.1	TRH (**1**)	1.0	1.0	1.0
9j	4.3	19.8	2.6				

a) Potency relative to TRH with 95% confidence limits. *b*) Increasing effect on spontaneous locomotor activity. *c*) Antagonistic effect on reserpine-induced hypothermia. *d*) Antagonistic effect on pentobarbital anesthesia. *e*) —=nonactive.

tetrahydroisoquinolines and related moieties were evaluated in mice by the methods described in the experimental section. Figures 1, 2 and 3 show the dose–response relationships for action on spontaneous locomotor activity, antagonistic effect on reserpine-induced hypothermia, and antagonistic effect on pentobarbital anesthesia of (3*S*)-Otc–His–Pro–NH_2 (**9a**) and TRH (**1**), respectively.

Thus, **9a** was approximately 10 times stronger in terms of increasing effect on spontaneous locomotor activity and in antagonistic effect on reserpine-induced hypothermia and 3.5 times more active in antagonistic effect on pentobarbital anesthesia than TRH. The relative potency ratios of **9a** and other test compounds **9b**—**q** and **13** with respect to TRH in each CNS action are summarized in Table II.

The CNS actions of the diastereomeric derivative **9b** of compound **9a**, which was anticipated to be resistant to enzymatic hydrolysis, were reduced to about half of those of **9a**. The analog **13** in which the Pro–NH_2 moiety is replaced by Tzl–NH_2 also showed markedly diminished effects. Then, we investigated substituent effects on the benzene ring of **9a**. In the

TABLE III. TSH-Releasing Activity of **9a** and TRH in Rats

Compd.	Dose μg/rat i.v.	Number of rats	Serum TSH (mean ± S.E.) μU/ml
Control	—	5	107 ± 38
9a	0.125	5	2743 ± 584
	2	5	3129 ± 912
TRH (**1**)	0.03125	5	267 ± 96
	0.125	5	904 ± 300
	0.5	5	3474 ± 857

case of electron-donating groups, the more hydrophylic 6,7-dihydroxy group was more effective than a 6,7-dimethoxy group from the evaluations of **9c** and **9d**. Among the substituents at the 7-position on Otc–OH, a more hydrophilic and basic amino group showed considerable effects (compound **9f**), whereas non-basic acylamino groups (compounds **9g** and **9h**) resulted in greatly diminished activities. The analogs **9e** and **9i** having electron-withdrawing nitro and chloro groups also exhibited stronger CNS actions than TRH, but were less potent than **9a**. These results suggested that introduction of substituents rather weakened the CNS actions. Interestingly, a methyl group at the 2-position showed relatively selective antagonism of reserpine-induced hypothermia, but had lesser effects on other actions (compound **9j**). From the viewpoint of bioisosteric character, the lactone-type peptides **9k**—**m** in which the nitrogen atom at the 2-position of Otc–OH is exchanged for oxygen were also tested. These compounds exhibited lower activities than the corresponding lactam-type peptide **9a**. Two analogs **9n** and **9o**, which were derived from the conjugated carboxylic acids **2n** and **2o**, did not show potent effects in comparison with the corresponding (*S*)-configurational saturated compounds **9a** and **9l**, respectively. The CNS actions of the tripeptides **9p** and **9q** having five-membered heterocycles fused with the benzene ring were also examined, but these effects were unexpectedly very weak and inferior to those of TRH.

In conclusion, many TRH analogs containing the tetrahydroisoquinoline moiety exhibited stronger CNS actions than TRH, and Otc–His–Pro–NH_2 (**9a**) showed the most potent CNS actions. It appears that the lipophilic phenyl ring contributes greatly to the enhancement of the CNS actions. Moreover, it was also shown that the size and configuration of the heterocycles fused with the benzene ring considerably influenced the CNS actions. The endocrine action of **9a** was also tested according to the known method,[22] but unfortunately, it was 3 to 4 times higher than that of TRH, as shown in Table III.

Consequently, it was found that the TRH analog **9a** is more potent in terms of both TSH-releasing activity and CNS actions than TRH. Further investigations are proceeding in an attempt to find the compounds with more potent CNS actions and less TSH-releasing activity.

Experimental

All melting points were measured by the use of a Yamato MP-21 melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a Shimadzu IR-420 spectrometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Hitachi R-40 (90 MHz) spectrometer using tetramethylsilane as an internal standard. The mass spectra (MS) were taken on a Hitachi M-60 spectrometer at an ionizing potential of 30 eV. Optical rotations were measured with a Perkin-Elmer 243 digital readout polarimeter using a 10 cm cell. For silica gel column chromatography, Kieselgel 60 (0.063—0.20 mm, E. Merck) was employed and for the purification of TRH analogs, MCI GEL CHP-20P (75—150 μm, Mitsubishi Chemical Industries) were utilized with aqueous MeOH as an eluent. TLC of TRH analogs was done on precoated plates Kieselgel $60F_{254}$ (E. Merck) in the following three solvent systems (v/v); Rf_1, *n*-BuOH–AcOH–AcOEt–H_2O (1 : 1 : 1 : 1); Rf_2, *n*-BuOH–AcOH–H_2O (4 : 1 : 1); Rf_3, dioxane–MeCN–H_2O–AcOEt–AcOH (18 : 12 : 6 : 2 : 1). The spots were detected under ultraviolet (UV) irradiation at 254 nm,

and by the use of Pauly reagent and Cl_2–tolidine color sprays. Electrophoresis on Toyo Roshi No. 51 paper was carried out with H_2O–pyridine–AcOH (95 : 1 : 10, v/v, pH 3.6) for 40 min at 2450 V/60 cm, and the spots were visualized with Pauly reagent spray. Analytical HPLC was performed on a Shimadzu LC-4A apparatus equipped with an SPD-2AS UV detector set at 210 nm using a column (4.6 × 150 cm) packed with Nucleosil $5C_{18}$ (Chemco Pak) at 40 °C with a solvent flow rate of 1.0 ml/min.

Chemistry

(3*S*)-2-Benzoyl-6,7-dihydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (5c)——Benzoyl chloride (27.5 g, 0.196 mol) and a solution of NaOH (13 g, 0.325 mol) in H_2O (100 ml) were simultaneously added dropwise to a suspension of **4c**[10] (33.9 g, 0.162 mol) and $Na_2B_4O_7 \cdot 10H_2O$ (62 g, 0.163 mol) in H_2O (500 ml) at 0 °C under stirring. After being stirred for 4 h at room temperature, the mixture was washed with AcOEt. The aqueous layer was acidified with concentrated HCl and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residual solids were triturated with benzene, collected by suction, and recrystallized from H_2O to give **5c** (41.7 g, 80%), mp 189—192 °C (dec.). $[\alpha]_D^{25}$ −12.0° (c = 1, MeOH). IR (Nujol): 1760, 1590 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.9—3.2 (2H, m, C4-H), 4.2—5.3 (3H, m, C1-H and C2-H), 6.3—6.8 (2H, m, C5-H and C8-H), 7.4—7.6 (5H, m, phenyl protons). MS m/z: 313 (M^+). *Anal.* Calcd for $C_{17}H_{15}NO_5 \cdot 1/4H_2O$: C, 64.24; H, 4.92; N, 4.41. Found: C, 64.45; H, 4.70; N, 4.50.

(3*S*)-2-Benzoyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (5d)——Dimethyl sulfate (134 g, 1.06 mol) and a solution of KOH (60.8 g, 0.922 mol) in H_2O (100 ml) were simultaneously added dropwise to a solution of **5c** (41.5 g, 0.133 mol) and KOH (9.0 g, 0.137 mol) in H_2O (100 ml) at 0 °C under stirring. The reaction mixture was stirred at room temperature for 2 h, and washed with AcOEt. The aqueous layer was acidified with concentrated HCl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo* to give **5d** as a syrup (32.5 g, 72%). IR ($CHCl_3$): 1720, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.9—3.4, (2H, m, C4-H), 3.78 (6H, s, $OCH_3 \times 2$), 4.2—5.7 (3H, m, C1-H and C2-H), 6.3—6.8 (2H, m, C5-H and C8-H), 7.4—7.6 (5H, m, phenyl protons), 8.92 (1H, s, CO_2H). MS m/z: 341 (M^+).

L-*N*-Benzoyl-(2-carboxy-4,5-dimethoxy)phenylalanine (6d)——Finely pulverized $KMnO_4$ (30.1 g, 0.191 mol) was gradually added to a solution of **5d** (32.5 g, 0.0953 mol) and K_2CO_3 (14.5 g, 0.105 mol) in H_2O (400 ml) at 20 °C and the mixture was stirred at room temperature overnight. Then, the unreacted $KMnO_4$ was reduced with $NaHSO_3$ (10 g) and stirring was continued for 2 h. MnO_2 was filtered off and the filtrate was acidified with concentrated HCl. The precipitate was collected by filtration and recrystallized from a mixture of acetone–H_2O (1 : 1) to afford **6d** (17.7 g, 50%), mp 241—243 °C (dec.). $[\alpha]_D^{25}$ −98.9° (c = 1, 1 N NaOH). IR (Nujol): 1755, 1685, 1635 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.0—3.9 (2H, m, Cβ-H), 3.81 (6H, s, $OCH_3 \times 2$), 4.7—5.0 (1H, m, Cα-H), 7.10 (1H, s, C2-H), 7.4—8.0 (6H, m, C5-H and phenyl protons), 8.88 (1H, br, NH). MS m/z: 373 (M^+). *Anal.* Calcd for $C_{19}H_{19}NO_7 \cdot 7/8H_2O$: C, 58.66; H, 5.38; N, 3.60. Found: C, 58.65; H, 5.11; N, 3.61.

(3*S*)-6,7-Dimethoxy-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2d)——A suspention of **6d** (14.5 g, 0.0389 mol) in 6 N HCl (900 ml) and AcOH (70 ml) was refluxed for 15 h. The reaction mixture was concentrated *in vacuo*. After the residue had been washed with benzene, the crude products were purified by column chromatography on silica gel ($CHCl_3$–MeOH–AcOH, 85 : 15 : 3). Recrystallization from a mixture of $CHCl_3$ and AcOH gave **2d** (1.2 g, 12%), mp 212—214 °C (dec.). $[\alpha]_D^{25}$ +32.7° (c = 1, MeOH). IR (Nujol): 3250, 1720, 1600 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.1—3.5 (2H, m, C4-H), 3.93 and 3.94 (3H each, 2s, $OCH_3 \times 2$), 4.2—4.5 (1H, m, C3-H), 6.77 (1H, s, C5-H), 7.56 (1H, s, C8-H), 8.1 (1H, br, NH). MS m/z: 251 (M^+). *Anal.* Calcd for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.21; N, 5.58. Found: C, 57.02; H, 5.14; N, 5.30.

Methyl (3*S*)-6,7-Dihydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (7c)——A suspension of **6d** (62 g, 0.166 mol) in AcOH (800 ml) and 47% aqueous HBr (60 ml) was refluxed for 15 h. The reaction mixture was concentrated to dryness *in vacuo*, and the residue was diluted with H_2O and washed with benzene. The aqueous layer was concentrated to dryness *in vacuo* again. MeOH was added to the residue and the mixture was refluxed for 2 h. The reaction mixture was concentrated *in vacuo* and the oily residue was chromatographed on silica gel ($CHCl_3$–MeOH–AcOH, 85 : 15 : 6). The crude product thus obtained was recrystallized from H_2O to give **7c** (12.0 g, 30%), mp 245—248 °C (dec.) $[\alpha]_D^{25}$ +19.8° (c = 1, MeOH). IR (Nujol): 3300, 1740, 1645 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.8—3.4 (2H, m, C4-H), 3.50 (3H, s, CH_3), 4.2—4.4 (1H, m, C5-H), 6.60 (1H, s, C5-H), 6.73 (1H, s, C8-H), 7.70 (1H, br, NH). MS m/z: 237 (M^+). *Anal.* Calcd for $C_{11}H_{11}NO_5$: C, 55.69; H, 4.68; N, 5.91. Found: C, 55.58; H, 4.65; N, 5.99.

(3*S*)-6,7-Dihydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2c)——A solution of NaOH (0.81 g, 20.3 mmol) and $Na_2B_4O_7 \cdot 10H_2O$ (7.08 g, 18.6 mmol) in H_2O (100 ml) were added to a solution of **7c** (4 g, 17 mmol) in MeOH (100 ml) at below 10 °C. The mixture was stirred at room temperature for 5 h, then the MeOH was removed *in vacuo*. The aqueous solution was acidified with 10 N HCl and the solution was passed through a column (2.6 × 35 cm) of MCI GEL CHP-20P. After washing of the column with H_2O (500 ml), the product was eluted with 50% aqueous MeOH and the eluate was concentrated *in vacuo*. The precipitate was collected by filtration and recrystallized from H_2O to give **2c** (1.81 g, 48%), mp 268—272 °C (dec.). $[\alpha]_D^{25}$ +0.7° (c = 1, DMF). IR (Nujol): 3300, 1730, 1600 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.8—3.4 (2H, m, C4-H), 4.0—4.3 (1H, m, C3-H), 6.61 (1H, s, C5-H), 7.28 (1H, s, C8-H), 7.55 (1H, br, NH), 8.5—9.6 (2H, br, OH × 2). MS m/z: 223 (M^+). *Anal.* Calcd for $C_{10}H_9NO_5 \cdot 9/8H_2O$: C, 49.33; H, 4.66; N, 5.75. Found: C, 49.38; H, 4.63; N, 5.85.

(3*S*)-7-Nitro-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2e)——Concentrated HNO_3 ($d=1.38$, 0.86 ml) was added to a solution of **2a** (1.0 g, 5.2 mmol) in concentrated H_2SO_4 ($d=1.84$, 4.5 ml) at -5 °C over a period of about 1 h. After being stirred for 30 min at 20 °C, the reaction mixture was poured into crushed ice (5 g) and the resulting crystals were collected by filtration, and washed with H_2O. This crude solid was recrystallized from MeOH to give **2e** (1.1 g, 90%), mp 255—256 °C (dec.). $[\alpha]_D^{25}$ +138.1° ($c=1$, DMF). IR (Nujol): 1715, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.1—3.6 (2H, m, C4-H), 4.2—4.4 (1H, m, C3-H), 7.51 (1H, d, $J=9$ Hz, C5-H), 8.17 (1H, dd, $J=9$, 3 Hz, C6-H), 8.40 (1H, d, $J=3$ Hz, C8-H). MS *m/z*: 237 ($M^+ +1$). *Anal.* Calcd for $C_{10}H_8N_2O_5$: C, 50.85; H, 3.41; N, 11.86. Found: C, 50.94; H, 3.36; N, 11.86.

(3*S*)-7-Amino-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2f)——Pd-black (1 g) was added to a suspention of **2e** (31 g, 0.131 mol) in MeOH (900 ml), and the mixture was subjected to catalytic hydrogenation under a hydrogen atmosphere for 2 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from H_2O to give **2f** (18.7 g, 69%), mp >300 °C. $[\alpha]_D^{25}$ +86.8° ($c=0.5$, 1 N HCl). IR (Nujol): 3480, 3380, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.7—3.3 (2H, m, C4-H), 3.9—4.2 (1H, m, C3-H), 6.53 (1H, dd, $J=8$, 3 Hz, C6-H), 6.82 (1H, d, $J=8$ Hz, C5-H), 6.99 (1H, d, $J=3$ Hz, C8-H), 7.59 (1H, br, NH). *Anal.* Calcd for $C_{10}H_{10}N_2O_3 \cdot 1/8H_2O$: C, 57.62; H, 4.96; N, 13.44. Found: C, 57.57; H, 4.92; N, 13.48.

(3*S*)-7-*tert*-Butyloxycarbonylamino-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2g)——Di-*tert*-butyl dicarbonate (1.1 g, 5.1 mmol) was added dropwise to a solution of **2f** (0.85 g, 4.1 mmol) in dioxane (8.5 ml) and 1 N NaOH (4.2 ml) at 10 °C and the mixture was stirred at room temperature overnight. The mixture was concentrated *in vacuo* to remove dioxane and the aqueous residue was washed with AcOEt. The separated aqueous layer was adjusted to pH 2 with 1 N H_2SO_4 and extracted with AcOEt. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from AcOEt to give **2g** (0.73 g, 56%), mp 195—196 °C (dec.). $[\alpha]_D^{25}$ +6.1° ($c=1$, MeOH). IR (Nujol): 3350, 3300, 1720 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.51 (9H, s, $CH_3 \times 3$), 2.9—3.5 (2H, m, C4-H), 4.1—4.4 (1H, m, C3-H), 7.21 (1H, d, $J=8$ Hz, C5-H), 7.55 (1H, dd, $J=8$, 3 Hz, C6-H), 7.97 (1H, br, NH), 8.09 (1H, d, $J=3$ Hz, C8-H), 9.45 (1H, s, CO_2H). *Anal.* Calcd for $C_{15}H_{18}N_2O_5$: C, 58.81; H, 5.92; N, 9.15. Found: C, 59.05; H, 5.90; N, 9.37.

(3*S*)-7-Acetamido-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2h)——Acetyl chloride (1.83 g, 23.3 mmol) and 1.5 N NaOH (28 ml) were simultaneously added dropwise to a solution of **2f** (4 g, 19.4 mmol) in 1.5 N NaOH (20 ml) at 0 °C under stirring. After being stirred for 30 min at the same temperature, the mixture was washed with AcOEt. The aqueous layer separated was acidified with 10% aqueous HCl and the resulting crystals were collected by filtration. Recrystallization from H_2O afforded **2h** (1.48 g, 31%), mp 278—282 °C (dec.). $[\alpha]_D^{25}$ +44.2° ($c=1$, DMF). IR (Nujol): 1725, 1670 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.98 (3H, s, NCH_3), 2.8—3.4 (2H, m, C4-H), 4.0—4.3 (1H, m, C3-H), 7.07 (1H, d, $J=8$ Hz, C5-H), 7.53 (1H, dd, $J=8$, 3 Hz, C6-H), 7.80 (1H, br, NH), 7.91 (1H, d, $J=3$ Hz, C8-H), 9.80 (1H, s, CO_2H). MS *m/z*: 248 (M^+). *Anal.* Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 57.89; H, 4.78; N, 11.24.

(3*S*)-7-Chloro-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2i)——$NaNO_2$ (0.306 g, 4.4 mmol) in H_2O (1 ml) was added dropwise to a suspension of **2f** (0.9 g, 4.4 mmol) in a mixture of concentrated HCl (1.1 ml) and H_2O (1 ml) at 0 °C over a period of 10 min under stirring. This yellow reaction mixture was poured into a solution of CuCl (0.64 g, 6.1 mmol) in concentrated HCl (2.6 ml) at 0 °C, and the mixture was heated 60 °C for 30 min with vigorous stirring. The resulting precipitate was collected and suspended in MeOH (100 ml), and the insoluble solid was collected by filtration. This crude product was dissolved in hot water. After cooling, the colored resinous matter was filtered off. The filtrate was concentrated *in vacuo* and the precipitate was collected to give **2i** (0.35 g, 36%), mp 252—254 °C (dec.). $[\alpha]_D^{25}$ +85.8° ($c=1$, DMF). IR (Nujol): 3300, 1720, 1640 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.8—3.5 (2H, m, C4-H), 4.0—4.4 (1H, m, C3-H), 7.21 (1H, d, $J=8$ Hz, C5-H), 7.41 (1H, dd, $J=8$, 3 Hz, C6-H), 7.65 (1H, d, $J=3$ Hz, C8-H), 8.05 (1H, br, NH). MS *m/z*: 225 (M^+). *Anal.* Calcd for $C_{10}H_8ClNO_3$: C, 53.23; H, 3.57; Cl, 15.72; N, 6.21. Found: C, 53.53, H, 3.58; Cl, 15.36; N, 6.42.

Methyl (3*S*)-2-Methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (8)——A mixture of **2a** (2.0 g, 10.5 mmol), silver oxide (7.28 g, 32 mmol) and methyl iodide (30 g, 0.21 mol) in DMF (50 ml) was stirred in the dark for 7 d. The insoluble material was filtered off and AcOEt was added to the filtrate. The mixture was washed with 30% aqueous $Na_2S_2O_3$, dried over $MgSO_4$, and concentrated *in vacuo*. The oily residue was chromatographed on silica gel (ether–$CHCl_3$, 7:3) to give **8** (2.1 g, 91%) as an oil. IR (film): 1740, 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.10 (3H, s, NCH_3), 3.2—3.5 (2H, m, C4-H), 3.45 (3H, s, OCH_3), 4.18 (1H, dd, $J=6$, 3 Hz, C3-H), 7.0—7.5 (3H, m, C5-H, C6-H, and C7-H), 7.9—8.1 (1H, m, C8-H). MS *m/z*: 219 (M^+).

(3*S*)-2-Methyl-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (2j)——A 1 N NaOH solution (10 ml) was added to a solution of **8** (2.05 g, 9.4 mmol) in MeOH (10 ml) at below 10 °C. The mixture was stirred at room temperature for 1 h, then the MeOH was removed *in vacuo* and the aqueous solution was acidified with 10% aqueous HCl, then extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was crystallized from MeOH to afford **2j** (1.02 g, 53%), mp 238—242 °C. $[\alpha]_D^{25}$ +171.8° ($c=1$ DMF). IR (Nujol): 1725, 1615 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.98 (3H, m, NCH_3), 3.0—3.6 (2H, m, C4-H), 4.38 (1H, dd, $J=7$, 3 Hz, C3-H), 7.0—7.4 (3H, m, C5-H, C6-H, and C7-H), 7.6—7.8 (1H, m, C8-H). MS *m/z*: 205 (M^+). *Anal.* Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.46; H, 5.33; N, 6.91.

Optical Resolution of 3,4-Dihydroisocoumarin-3-carboxylic Acid (2k)——Racemic acid **2k**[12] (4.8 g, 25 mmol) and D-Phe–NH_2 (4.1 g, 25 mmol) were dissolved in 2-propanol (400 ml) at 80 °C. The mixture was gradually cooled to room temperature and the precipitate that separated was collected by filtration. Recrystallization from 2-propanol gave the salt; (3*S*)-isomer (**2l**)·D-Phe–NH_2 (1.8 g), mp 186—188 °C. $[\alpha]_D^{25}$ −2.2° (*c*=1, MeOH). *Anal.* Calcd for $C_{19}H_{20}N_2O_5$: C, 64.03; H, 5.66; N, 7.86. Found: C, 64.12; H, 5.61; N, 7.86. The salt was dissolved in H_2O, acidified with 10% aqueous HCl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated to dryness *in vacuo*. The residue was recrystallized from a mixture of AcOEt and hexane to afford the (3*S*)-isomer (**2l**) (0.8 g, 17%), mp 174—177 °C. $[\alpha]_D^{25}$ +52.2° (*c*=1, MeOH), lit.[17] $[\alpha]_D^{27}$ +44° (*c*=1.24, MeOH). IR (Nujol): 1760, 1700 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.27 and 3.55 (1H each, 2dd, *J*=18, 5 Hz, C4-H), 5.35 (1H, t, *J*=5 Hz, C3-H), 7.3—7.8 (3H, m, C5-H, C6-H, and C7-H), 7.9—8.1 (1H, m, C8-H). MS *m/z*: 192 (M^+). *Anal.* Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.19. Found: C, 62.39; H, 4.19.

The combined 2-propanol mother liquor was concentrated *in vacuo* to give a crude residue, which was dissolved in H_2O. This solution was acidified with 10% aqueous HCl, and extracted with AcOEt. The extract was dried over $MgSO_4$, and concentrated *in vacuo*, then the resulting residue was dissolved in a solution of L-Phe–NH_2 (2.92 g, 17.8 mmol) in 2-propanol (350 ml) at 80 °C. The mixture was gradually cooled to room temperature and the precipitate was collected to give the salt; (3*R*)-isomer (**2m**)·L-Phe–NH_2 (2.82 g), mp 184—187 °C. $[\alpha]_D^{25}$ +1.0° (*c*=1, MeOH). *Anal.* Calcd for $C_{19}H_{20}N_2O_5$: C, 64.03; H, 5.66; N, 7.86. Found: C, 64.29; H, 5.59; N, 7.80. In the same manner as mentioned above, the salt was converted into the (3*R*)-isomer (**2m**) (1.43 g, 30%), mp 173—177 °C. $[\alpha]_D^{25}$ −51.0° (*c*=1, MeOH), lit.[17] $[\alpha]_D^{27}$ −43° (*c*=1.31, MeOH). Spectroscopic data of this compound were in good accordance with those of **2k** and **2l**. *Anal.* Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.19. Found: C, 62.47; H, 4.13.

Typical Example of the Preparation of TRH Analogs (9a)——DCC (1.2 g, 6 mmol) was added to a solution of **2a** (956 mg, 5 mmol) and HONSu (633 mg, 5.5 mmol) in DMF (14 ml) at 0 °C and the stirring was continued for 1 h. His–Pro–NH_2·2HBr (1.66 g, 5 mmol) and triethylamine (1.01 g, 10 mmol) were added to the mixture at 0 °C, and the whole was stirred at 10 °C for 48 h, then filtered to remove DCU. The filtrate was concentrated *in vacuo*. The residue was taken up in H_2O and the mixture was filtered again. The filtrate was adjusted to pH 8 by adding $NaHCO_3$ and passed through a column (3×35 cm) packed with MCI GEL CHP-20P. After washing of the column with H_2O (500 ml), the product was eluted with 30% aqueous MeOH. The fractions containing the desired product were collected and concentrated *in vacuo*. The residue was recrystallized from H_2O to give **9a** (1.07 g). IR (Nujol): 3280, 1640 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.6—2.1 (4H, m, Proβ-CH_2 and γ-CH_2), 2.7—4.4 (9H, m), 4.4—4.8 (1H, m, Hisα-CH), 6.72 and 7.52 (1H each, 2s, imidazole CH), 7.2—8.2 (4H, m, phenyl protons). MS *m/z*: 424 ($M.^+$). For the analytical HPLC of this compound and diastereomeric (3*R*)-Otc–His–Pro–NH_2 (**9b**), 0.05 M KH_2PO_4 (pH 2.5) +0.1% (w/v) sodium 1-pentanesulfonate : MeCN (9 : 1, v/v) was used as an eluent. Retention times were 6.66 and 7.30 min for the former and the latter, respectively. In a similar way, other TRH analogs (**9b—e**, **9g—q**, and **13**) were prepared. The yields and physicochemical data are listed in Table I.

Reaction of 9g with TFA: (3*S*)-7-Amino-Otc–His–Pro–NH_2 (9f)——Compound **9g** (710 mg, 1.3 mmol) was added to a mixture of anisole (0.5 ml) and TFA (5 ml) at 0 °C and the solution was stirred for 30 min. The mixture was concentrated *in vacuo*. The residue was taken up in H_2O and ether, and the aqueous layer was separated. The pH was adjusted to 8 by adding $NaHCO_3$, and the solution was passed through a CHP-20P column (3×35 cm). The column was washed with H_2O (500 ml) and the product was eluted with 30% aqueous MeOH. The fractions containing the desired product were collected, concentrated *in vacuo* to a small volume, and lyophilized to give **9f** (570 mg) as an amorphous substance. IR (Nujol): 3300, 1640. ^{1}H-NMR (D_2O) δ: 1.6—2.1 (4H, m, Proβ-CH_2 and γ-CH_2), 2.7—3.6 (6H, m), 4.1—4.7 (3H, m, Proα-CH, Hisα-CH, and OtcC3-H), 6.99 and 8.50 (1H each, 2d, *J*=1 Hz, imidazole CH), 7.25 (1H, d, *J*=9 Hz, OtcC5-H), 7.40 (1H, dd, *J*=9, 3 Hz, OtcC6-H), 7.71 (1H, d, *J*=3 Hz, OtcC8-H).

Nps–His(Tos)–Tzl–NH_2 (11)——DCC (1.7 g, 8.3 mmol) was added to a mixture of Nps–His(Tos)–OH[20a] (**10**; 3.1 g, 6.7 mmol) and Tzl–NH_2[5b] (1.06 g, 6.7 mmol) in THF (30 ml) and DMF (5 ml) at 0 °C. The whole was stirred at room temperature overnight, then filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in AcOEt. After washing of the solution with 1 N H_2SO_4 and saturated $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$ and the solution was concentrated *in vacuo*. The crude solid thus obtained was recrystallized from MeOH to afford **11** (1.8 g, 47%), mp 180—182 °C, $[\alpha]_D^{25}$ −36.2° (*c*=1, DMF). IR (Nujol): 3450, 3150, 1700, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.43 (3H, s, CH_3), 2.8—3.2 (4H, m, Hisβ-CH_2 and Tzlβ-CH_2), 3.9—5.2 (4H, m, Hisα-CH, Tzlα-CH, and SCH_2N), 7.0—8.4 (10H, m, aromatic protons). *Anal.* Calcd for $C_{23}H_{24}N_6O_6S_3$: C, 47.90; H, 4.19; N, 14.57; S, 16.68. Found: C, 47.75; H, 4.11; N, 14.55; S, 16.40.

His–Tzl–NH_2·2HCl (12)——Compound **11** (670 mg, 1.16 mmol) was dissolved in a mixture of DMF (2 ml) and MeOH (200 ml), and the mixture was cooled to 0 °C. After being saturated with NH_3 gas, the solution was allowed to stand at room temperature overnight in a sealed container. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in 10% aqueous citric acid solution. The solution was washed with AcOEt and the aqueous layer was adjusted to pH 8 by adding $NaHCO_3$. The alkaline solution was extracted with AcOEt, dried over $MgSO_4$, and concentrated to give an oily residue. The residue was dissolved in dioxane (3 ml) and 25% HCl–MeOH (600 mg, 4 mmol) was added dropwise to the solution. The mixture was stirred for 5 min, then ether (10 ml) was added. The solid that precipitated was collected by filtration, dried in a desiccator under reduced pressure for a day, and used

immediately for the preparation of **13**.

Pharmacology

Materials——Synthetic TRH[20c] was used as a reference drug in all biological tests. Other drugs used were as follows: reserpine (Nakarai Chemicals), pentobarbital Na (Nakarai Chemicals).

Effect on Spontaneous Locomotor Activity[4a]——Four or five male Std/ddY mice (26—28 g) were used in each group. Mice were individually placed in Ambulometer (AMB-10, Ohara Ika) for 30 min to acclimatize them to the apparatus. Test compounds dissolved in physiological saline or physiological saline alone as a control were intraperitoneally administered to the mice. Spontaneous locomotor activity was measured as the total counts for 1 h after administration of a test compound. Relative potency ratio of each test compound with respect to TRH was calculated from the total counts at each dose of the test compound and TRH by the parallel line assay method.

Effect on Reserpine-Induced Hypothermia[4a]——Five male Std/ddY mice (26—28 g) were used in each group. Test compounds dissolved in saline or saline alone were intraperitoneally administered to mice about 18 h after subcutaneous treatment with 3 mg/kg of reserpine. Rectal temperature was measured with a thermister (MGA-III, Nihon Koden) 30 min before and 15, 30, 60, 120, and 180 min after administration of a test compound. Relative potency ratio of each test compound with respect to TRH was calculated from the area under the curve at each dose of the test compound and TRH by the parallel line assay method.

Effect on Pentobarbital Anesthesia[4b]——Six male Std/ddY mice (26—28 g) were used in each group. Test compounds dissolved in saline or saline alone were intravenously administered to mice with loss of the righting reflex 10 min after intraperitoneal administration of 55 mg/kg of pentobarbital Na. The duration of anesthesia was measured as the time from the end of intravenous administration of a test compound until the recovery of righting reflex. The relative potency ratio of each test compound with respect to TRH was calculated from the duration of anesthesia at each dose of the test compound and TRH by the parallel line assay method.

TSH-Releasing Activity[22]——Five male Jcl : SD rats (180—220 g, 5 weeks old) were used in each group. Test compounds dissolved in physiological saline containing 0.1% BSA or BSA-containing physiological saline as a control were intravenously administered to rats in a volume of 0.5 ml per rat. Blood samples were taken from the abdominal aorta under pentobarbital anesthesia and serum TSH levels were determined by the double-antibody radioimmunoassay method.

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References and Notes

1) *a*) This work was presented at the 36th Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Osaka, November 1986; *b*) Amino acids and their derivatives, unless otherwise designated, are L. Symbols and nomenclature follow the recommendations published by the IUPAC-IUB Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.*, **138**, 9 (1984). The following abbreviations are used: DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; HONSu, *N*-hydroxysuccinimide; Nps, 2-nitrophenylthio; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; BSA, bovine serum albumin.

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