

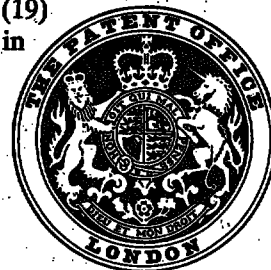
PATENT SPECIFICATION

(11)

1 579 264

1 579 264

- (21) Application No. 12204/77 (22) Filed 23 Mar. 1977 (19)
 (31) Convention Application No. 51/032170 (32) Filed 23 Mar. 1976 in
 (33) Japan (JP)
 (44) Complete Specification Published 19 Nov. 1980
 (51) INT. CL.³ C07C 103/52
 A61K 37/02

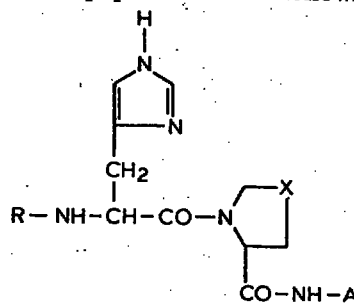


(52) Index at Acceptance

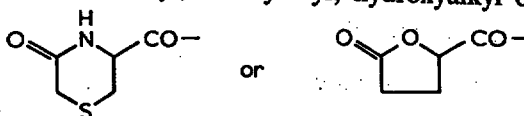
C3H	302	303	350	A3
C2C	1341	1472	1532	1582
	215	220	226	
	22Y	247	250	251
	253	256	25Y	271
	280	281	305	30Y
	342	34Y	351	352
	366	367	368	387
	401	40Y	574	579
	584	594	625	628
	62X	65X	678	699
	761	762	767	AA
				KF
				KK
				TR
				TV

(54) PEPTIDES

(71) We TAKEDA YAKUHIIN KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES LTD. a company organised and existing under the laws of Japan of 27 Doschomachi 2-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:
 This invention relates to novel peptides of the following formula:



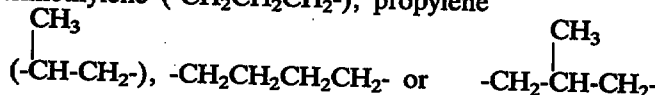
wherein A is hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy; R is



X is -S- or $-(CH_2)_n$ - where n is 1 or 2; and R and other constituent aminoacid residues may each be of L-, D- or DL- configuration.

This invention provides firstly novel peptides of the formula (I), and also peptides (I) which, whilst being weaker than the known TRH (L-pyroglutamyl-L-histidyl-L-prolinamide) in TSH (thyroid stimulating hormone)-releasing activity, are stronger than the known TRH in at least one of anaesthesia-antagonistic activity, spontaneous movement-stimulant activity and dopamine-potentiating activity.

In the above formula (I), the alkyl group represented by A is preferably a straight-chain or branched alkyl group of up to 10 carbon atoms (e.g. methyl, ethyl, propyl, iso-propyl, butyl, sec-butyl, tert-butyl, iso-butyl, amyl, hexyl, octyl, nonyl or decyl). The aralkyl group represented by A is preferably a group consisting of a phenyl group to which a straight-chain or branched alkylene group of 1 to 4 carbon atoms (e.g. methylene, ethylene, 1,3-trimethylene $-(CH_2CH_2CH_2)-$), propylene

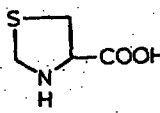
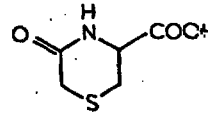
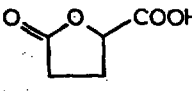


is attached. The alkoxyalkyl group represented by A is preferably a straight-chain or branched group of up to 9 carbon atoms (e.g. methoxymethyl, methoxyethyl, propoxyp-
 5 ropyl, butoxybutyl or methoxyoctyl). The alkoxy group represented by A preferably contains up to 9 carbon atoms, being exemplified by alkoxy groups corresponding to the alkyl groups A mentioned above.

The hydroxyalkyl preferably contains 1 to 9 carbon atoms. As examples thereof, there may be mentioned the alkyl groups of 1 to 9 carbon atoms mentioned for A which have been substituted by hydroxyl in any optional position.

Throughout this specification, the amino acids, peptides, compound residues, protective groups and solvents, are sometimes designated by abbreviations according to the IUPAC-IUB Commission on Biological Nomenclature or by the trivial names commonly
 10 used in the art.

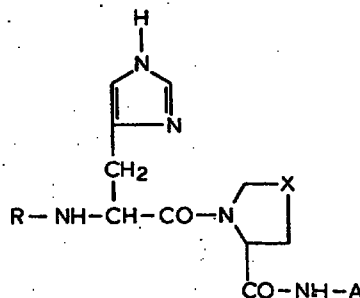
The following is a partial list of those abbreviations and trivial names.

15	His:	histidine	15
	Pro:	proline	
	Pip:	pipecolic acid	
20	Glu:	glutamic acid	20
	Tac:	3-thiazolidine-5-carboxylic acid	
25			25
30	Oct:	3-oxo-5-carboxy-perhydro-1,4-thiazine	30
			
35			35
	Cbl:	γ-carboxy-γ-butyrolactone	
40			40
	Z:	benzyloxycarbonyl	
45	BOC:	t-butoxycarbonyl	45
	DCC:	dicyclohexylcarbodiimide	
	N ₃ :	azide	
50	O ^t Bu:	t-butyl ester	50
	Tos:	tosyl	
55	HONB:	N-hydroxy-5-norbornene-2,3-dicarboximide	55
	Hobt:	N-hydroxy-1,2,3-benzotriazole	
	Hosu:	N-hydroxysuccinimide	
60	DCHA:	dicyclohexylamine	60
	DMF:	dimethylformamide	

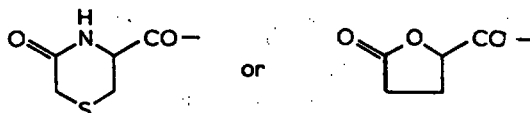
The above abbreviations may stand for the residues of the corresponding compounds capable of forming a peptide bond.

The contemplated compounds (I) of this invention may be produced by procedures known for the synthesis of peptides. The introduction of protective groups, the manner of forming peptide linkages, the removal of protective groups, etc. may be conventional *per se*. The compounds (I) may be produced either in liquid phase or in solid phase. The processes for peptide synthesis *per se* which may be employed for the production of compounds (I) are described in, for example, "The Peptides", Vol. 1 (1966), Schröder and Lubke, Academic Press, New York, U.S.A.; "Amino Acids, Peptides and Proteins", Vols. 1-5, ed. by G.T. Young, published by The Chemical Society, London; "Peptide Synthesis" by Nobuo Izumiya (Maruzen); and United States Patent No. 3870694 issued to Fujino *et al.* Thus, there may be mentioned for example, the DCC/HONB process, the azide process, the chloride process, the acid anhydride process, the mixed anhydride process, the DCC process, the activated ester process, the process employing Woodward's Reagent K. carbodiimidazole process, the redox process and the EEDQ (1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) process.

According to our invention we provide a process for producing a compound of the formula (I):

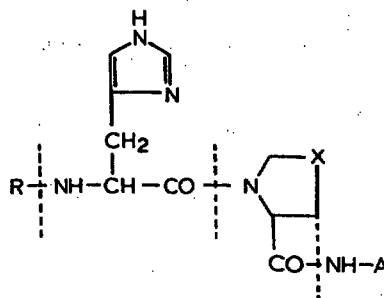


wherein A is hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy; R is



and X is -S- or $-(CH_2)_n-$ where n is 1 or 2, and R and other constituent amino acid residues may each be of L-, D- or DL-configurations, or a pharmacologically acceptable salt thereof with an inorganic or organic acid, in which an amino acid or a peptide fragment constituting one component of the compound (I) is reacted with the fragment constituting the remaining component of the compound (I), any group or groups not involved in amide bond formation being protected by blocking if necessary and later unblocked if necessary and/or any group or groups which take part in said formation being activated if necessary, and, if desired the resulting compound (I) is reacted with an inorganic acid or an organic acid yielding therewith the desired pharmacologically acceptable salt.

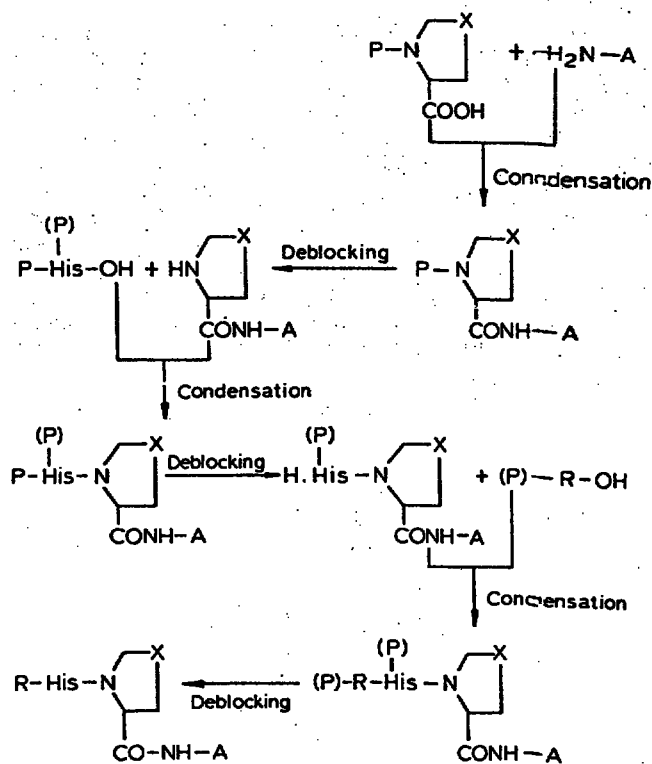
In the production of the contemplated compound (I) of this invention, the starting materials corresponding to the two portions divided by one of the following three dotted lines may be reacted together in a manner which is conventional *per se*.



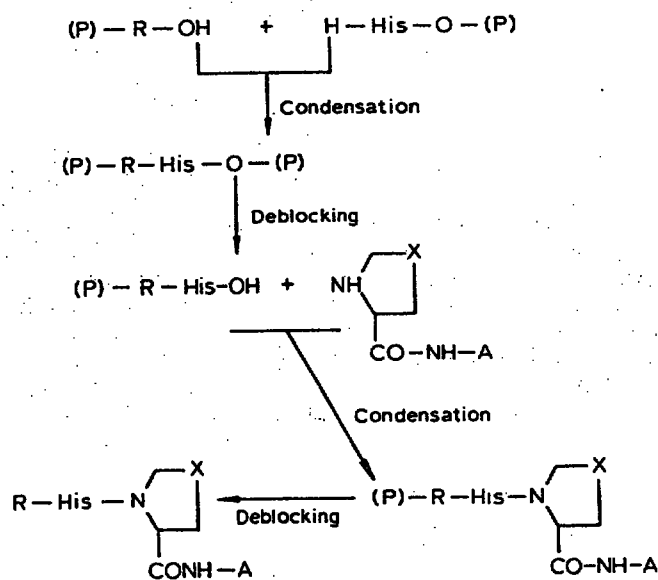
13

The following are typical examples of the production process.

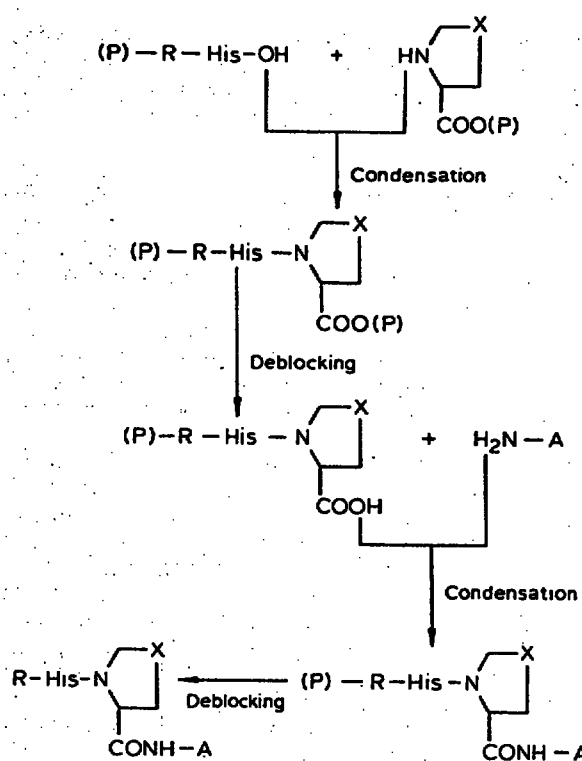
(1)



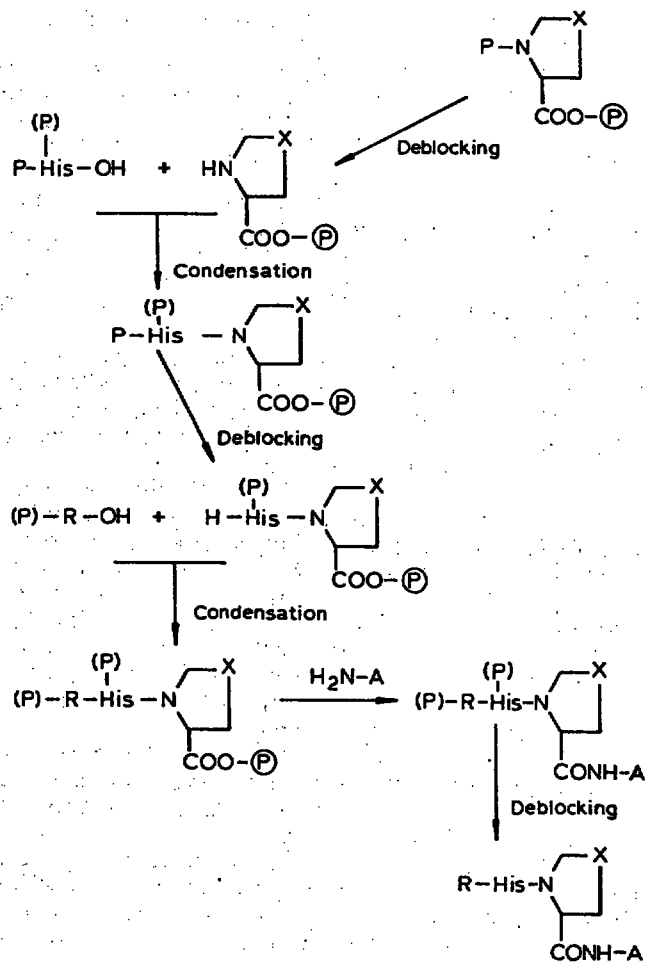
(2)



(3)



(4)



In the above formulae, P is a protective group; by (P) is meant that a protective group is not necessarily required; $\textcircled{P}$ is a resin for use in solid-phase synthesis; by the term 'condensation' is meant not only condensation by means of a dehydrative condensing agent but also condensation *via* an intermediate, e.g. the azide or chloride or an activated ester thereof.

Prior to the reaction for the formation of a peptide bond in the production of the contemplated compound (I), the functional groups such as amino, imino and carboxyl which will or ought not to be involved in the reaction may be protected with protective groups in known manner. The amino, imino and carboxyl groups pertinent to the peptide-forming reactions may be activated by known activating procedures.

The protective group for the α -amino group of any starting material (e.g. the α -amino group of histidine), which does not take part in the reaction, may be, for example, a conventional protective group such as Z, BOC, *t*-amyloxycarbonyl, isobornyloxycarbonyl, phthaloyl, trifluoroacetyl or formyl. The protective group for the imidazole nucleus of the histidine moiety may be a known group, for example, tosyl, benzyl or 2,4-dinitrophenyl, although such protection may not be essential.

The carboxyl group of the starting compound, which does not take part in the contemplated reaction, may be previously protected with a known protective group. Thus, for example, it may be protected in the form of an ester (e.g. methyl, ethyl, benzyl, *p*-nitrobenzyl, *t*-butyl or *t*-amyl ester), or a metal salt (e.g. the sodium or potassium salt).

The carboxyl group which will be involved in the contemplated reaction may be previously activated in such known forms as an activated ester (pentachlorophenol, 2,4,5-trichlorophenyl, 2,4-dinitrophenol, cyanomethyl alcohol, *p*-nitrophenol, N-hydroxy-5-norbornene-2,3-dicarboximide, N-hydroxysuccinimide, N-hydroxyphthalimide or an N-hydroxy-1,2,3-benzotriazole ester) or a carboxylic anhydride or azide corresponding to the starting material carboxylic acid. Among the above-mentioned activated esters, N-hydroxy-5-norbornene-2,3-dicarboximide, N-hydroxy-1,2,3-benzotriazole and N-hydroxysuccinimide are in certain cases more advantageous for the condensation reaction of the histidine moiety because of their relatively reduced tendency toward inducing racemization.

The present condensation reaction may be conducted in a solvent which does not interfere with the desired reaction, e.g. DMF, chloroform, dioxane or tetrahydrofuran.

The reaction temperature and time may be chosen from the ranges employed in conventional peptide synthesis, and they are preferably from -30°C . to 60°C . and from 2 to 24 hours.

Where the peptide produced by this condensation process has a protective group, the protective group can be removed by a conventional procedure. As examples of such deblocking procedure, there may be mentioned catalytic reduction with a catalyst (e.g. palladium black, palladium-on-carbon or platinum), solvolysis by means of an acid reagent (e.g. hydrogen fluoride, hydrogen bromide, hydrogen chloride or tri-fluoroacetic acid) and reduction with sodium in liquid ammonia.

At the end of the reaction, the product compound (I) may be separated from the reaction mixture as the free compound, or in the form of an acid salt, by procedures known *per se* (e.g. phasic transfer, extraction, chromatography, crystallization or reprecipitation).

The starting materials for the production of compounds (I) can also be prepared by known procedures or by procedures analogous thereto.

The product compounds (I) are able to form salts with pharmacologically acceptable inorganic acids (e.g. hydrochloric acid) or organic acids (e.g. acetic acid or tartaric acid).

The product compounds (I) according to this invention are of value as they display at least one of anaesthesia antagonism, sleep antagonism, spontaneous movement-stimulating activity and dopamine-potentiating activity in animals (e.g. mouse, rat, cat, dog or monkey). Furthermore, since these compounds have no or little TSH-releasing activity, they are of value in this respect as well. Of the Compound (I), the L-isomer is most desirable and the racemic mixture is second. Compounds (I) may be administered to animals (e.g. mouse, rat, cat, dog or monkey) or to human beings as paresthesias, spontaneous movement stimulants or dopamine-potentiators.

Compounds (I) are also useful in the treatment of hypnotic intoxication, clouding of consciousness, hyperkinesia, schizophrenia, depression and parkinsonism.

The possible routes of administration are by parenteral (e.g. intravenous, intramuscular or subcutaneous), oral, rectal, nasal or other routes.

The dosage of compound (I) necessary to attain the above-mentioned effects depends upon the particular species of compound (I), the species and health of animal, the route of administration, etc. For example, one may choose an appropriate dose from the range of 0.1 mg/kg to 10 mg/kg (per dose) in the case of parenteral administration, and from the range of 5 mg/kg to 500 mg/kg (per dose) for oral use.

The invention accordingly also covers a pharmaceutical composition comprising one or more compounds according to the invention together with a pharmaceutically acceptable carrier or diluent therefor.

While compounds (I) may be administered as they are, they may be formulated into dosage forms similar to those of known TRH (e.g. injectable preparations, powders or tablets).

The invention is illustrated by the following examples.

In all the examples and pharmacological test data given hereinafter, all references to amino acids, peptides and other compounds involving optical isomers are made to L-compounds unless otherwise indicated.

EXAMPLE 1

Synthesis of Oct-His-Pro-NH₂

(a) Synthesis of 3-oxo-5-carboxy-perhydro-1,4-thiazine

In 40 ml of water are suspended 3.6 g (0.02 M) of S-carboxymethyl-L-cysteine, followed by the addition of 20 ml of 1N-NaOH. The reaction is conducted in a sealed tubular reactor at 110°C for 48 hours. The reaction mixture is passed through a column (4.5 × 12.0 cm) of Amberlite (Trade Mark) IR-120 B (H⁺-form), whereby it is desalted. The effluent is concentrated to dryness and the residue is dissolved in glacial acetic acid. Following the addition of diethyl ether, the resulting crystals are recovered by filtration. Yield: 1.55 g. (48.1 mole%); melting point: 152.0-153.0°C; optical rotation: $[\alpha]_D^{25} + 1.1^\circ$ (c=0.64, in water).

Elemental analysis, for C₅H₇O₃NS:

Calcd.	C, 37.26; H, 4.38; N, 8.69; S, 19.89
Found	C, 36.97; H, 4.28; N, 8.54; S, 19.73

(b) Synthesis of Z-His-Pro-NH₂

With palladium black as the catalyst, 32.7 g (0.12 M) of Z-Pro-NH₂ are catalytically reduced in methanol in the conventional manner. The catalyst is filtered off and the filtrate is concentrated under reduced pressure. The resulting crystalline residue is thoroughly dried *in vacuo*. Separately, 36.4 g. (0.12 M) of Z-His-NHNH₂ are dissolved in 360 ml of INHCl and, after 480 ml. of ethyl acetate have been added, the solution is cooled to 0°C. With vigorous stirring, an aqueous solution (30 ml) of 8.3 g (0.12 M) of sodium nitrite is added dropwise. After 3 minutes, 144 ml of a cold 50% (W/V) solution of potassium carbonate are added. The ethyl acetate layer is taken and the water layer is extracted with ethyl acetate. Both extracts are pooled and dried over anhydrous Na₂SO₄. This dried ethyl acetate solution is combined with the dry powder previously obtained, followed by vigorous stirring. To this reaction mixture are added 150 ml of N,N-dimethylformamide and the mixture is stirred at 4°C for 12 hours, at the end of which time the solvent is distilled off. The oily residue is crystallized by the addition of cold water. It is further recrystallized from water. Yield: 24.5 g (53.0 mole%); melting point: 112.0-118.0°C; optical rotation: $[\alpha]_D^{22} - 39.8^\circ$ (c=0.53, in water).

Elemental analysis, for C₁₉H₂₃N₅O₄·1/2H₂O:

Calcd.	C, 57.86; H, 6.13; N, 17.76
Found	C, 58.16; H, 6.03; N, 17.65

(c) Synthesis of Oct-His-Pro-NH₂

In 15 ml of 25% (W/V) HBr/acetic acid are dissolved 1.20 g (0.00312 M) of Z-His-Pro-NH₂ and the solution is allowed to stand at room temperature for 40 minutes. Following the addition of 150 ml of diethyl ether, the resulting precipitate is recovered by filtration and dried in a desiccator with NaOH for 12 hours. The dried powder is dissolved in 20 ml of N,N-dimethylformamide and, under cooling, 0.88 ml (0.00624 M) of triethylamine is added. The resulting salt crystals are moved, and 502 mg (0.00312 M) of 3-oxo-5-carboxyperhydro-1,4-thiazine and 670 mg (0.00374 M) of HONB are dissolved in the filtrate. Then, at 0°C, 772 mg (0.00374 M) of DCC are added, followed by stirring in a refrigerator for 48 hours. The resulting byproduct dicyclohexylurea is filtered off and the filtrate is concentrated *in vacuo*. To the oily product thus obtained is added ethyl acetate and the resulting precipitate is dissolved in 70 ml of a solvent mixture of methanol and chloroform (1:9 by volume). The solution is run onto a column (4.5 × 6.0 cm) of silica gel and, following the passage of 300 ml of the same solvent as above, elution is carried out with a mixture of methanol and chloroform (4:6 by volume). The fractions rich in the desired product are pooled and, after the removal of the solvent by distillation, the oily residue is dissolved in water. The insolubles are filtered off and the filtrate is freeze-dried. Yield: 771

mg; optical rotation: $[\alpha]_D^{25} -57.5^\circ$ ($c=0.53$, in water); thin-layer chromatography: R_f , (n-butanol-ethyl acetate-acetic acid-water = 1:1:1:1 by volume, support = silica gel, Pauly's colour reaction) 0.34.

- 5 Elemental analysis, for $C_{16}H_{22}O_4N_6S \cdot 5H_2O$ 5
 Calcd. C, 39.67; H, 6.65; N, 17.36; S, 6.62
 Found C, 39.39; H, 5.12; N, 17.43; S, 6.79

EXAMPLE 2.

10 Synthesis of Oct-His-Pip-NH₂ 10

(a) Synthesis of Z-Pip-NH₂

- In 50 ml of a mixture of dioxane and ethyl acetate (4:1 by volume) are dissolved 3.95 g (0.015 M) of Z-Pip-OH and 2.70 g (0.015 M) of HONB. Then, at $0^\circ C$, 3.09 g (0.015 M) of DCC are added. The mixture is stirred at room temperature for 5 hours. The by-product dicyclohexylurea is filtered off and 5 ml of concentrated aqueous ammonia are added dropwise to the filtrate with vigorous stirring. After 5 hours, the solvent is distilled off under reduced pressure and the residue is dissolved in ethyl acetate. The ethyl acetate layer is washed with 5% (W/V) $NaHCO_3$ and $INHCl$, followed by drying. The ethyl acetate is distilled off and the residue is crystallized by the addition of petroleum benzene. The product is further recrystallized from ethyl acetate-petroleum benzene. Yield: 2.62 g (66.7 mole%); melting point: $110.0-111.0^\circ C$; optical rotation: $[\alpha]_D^{26} -36.1^\circ$ ($c = 0.42$ in methanol).

Elemental analysis, for $C_{14}H_{18}O_3N_2$:

- 25 Calcd. C, 64.10; H, 6.92; N, 10.68 25
 Found C, 64.11; H, 6.90; N, 10.67

(b) Synthesis of Z-His-Pip-NH₂

- In a routine manner, 5.3 g (0.02 M) of Z-Pip-NH₂ were catalytically reduced to H-Pip-NH₂. Separately, 6.1 g (0.02 M) of Z-His-NHNH₂ are converted to the azide as set in Example 1 (b), and are reacted with a solution of H-Pip-NH₂ in 100 ml of DMF at $4^\circ C$ for 4 days and at room temperature for 24 hours. The solvent is distilled off and the residue is dissolved in a solvent mixture of Rf_2 (ethyl acetate-pyridine-acetic acid-water = 60:20:6:11 by volume), and purified by chromatography on silica gel (column: 3.0×20.0 cm) with the same solvent system as above. The fractions rich in the desired product are pooled and concentrated to obtain an oily product. Yield: 4.0 g (50.0 mole%); optical rotation: $[\alpha]_D^{21} -48.6^\circ$ ($c=0.54$, in DMF).

Elemental analysis, for $C_{20}H_{25}O_4N_5 \cdot H_2O$:

- 40 Calcd. C, 57.54; H, 6.52; N, 16.78 40
 Found C, 57.47; H, 6.48; N, 15.46

(c) Synthesis of Oct-His-Pip-NH₂

- 1.20 g (0.003 M) of oily Z-His-Pip-NH₂ are treated with 12 ml of 25% (W/V) HBr /acetic acid for 110 minutes, at the end of which time 100 ml of diethyl ether are added. The resulting precipitate is recovered by filtration and dried in a desiccator with $NaOH$ for 12 hours. The dried powder is dissolved in 15 ml of N,N -dimethylformamide and the solution is neutralized with 0.84 ml (0.0006 M) of triethyl-amine under cooling. The precipitated salt is filtered off and the filtrate is directly used as the amine component for the next reaction. 484 mg (0.003 M) of 3-oxo-5-carboxyperhydro-1,4-thiazine and 540 mg (0.003 M) of HONB are dissolved in 5 ml of N,N -dimethylformamide, and, under ice-cooling, 618 mg (0.003 M) of DCC are added. The mixture is stirred for 3 hours. The activated ester solution is combined with the amine component solution prepared above and the entire mixture is stirred at $4^\circ C$ for 12 hours. The by-product dicyclohexylurea is filtered off and the filtrate is concentrated *in vacuo*. The oily residue is triturated with diethyl ether. The powder thus obtained is dissolved in 100 ml of a mixture of methanol and chloroform (1:9 by volume) and passed through a column (2.5×16.0 cm) of silica gel. The column is first washed with 700 ml of the same solvent as above and eluted with a mixture of methanol and chloroform 4:6 by volume. The fractions rich in the desired compound are pooled and concentrated under reduced pressure to remove the solvent. The residue is dissolved in a small amount of 0.1N acetic acid and further purified by means of a column (5.3×40.0 cm) of Sephadex LH-20 (Trade Mark). The fractions rich in the compound are pooled and freeze-dried. Yield: 740 mg; optical rotation: $[\alpha]_D^{21} -52.3^\circ$ ($c=0.70$, in water); thin-layer chromatography: $R_f=0.50$.

Elemental analysis, for $C_{17}H_{24}O_4N_6S \cdot CH_3COOH \cdot 4H_2O$

Calcd. C, 42.21; H, 6.71; N, 15.55; S, 5.93

Found C, 41.94; H, 5.38; N, 16.00; S, 6.48

Aminoacid analysis (hydrolysed in 6N HCl at 110°C for 24 hours): His, 0.93(1); s-carboxymethylcysteine, 1.03(1); Pip, 1.00(1); average recovery: 94.2 mole%.

EXAMPLE 3

Synthesis of Oct-His-Pro-NHCH₃

(a) *Synthesis of Z-Pro-NH-CH₃*

In 100 ml of dioxane there are dissolved 12.5 g (0.05 M) of Z-Pro-OH together with 9.8 g (0.055 M) of HONB and, under cooling, 11.3 g (0.055 M) of DCC are added. The mixture is stirred at room temperature for 3 hours and the by-product dicyclohexylurea is filtered off. The filtrate is stirred vigorously with 5.06 g (0.075 M) of methylamine hydrochloride, 10.5 ml (0.075 M) of triethylamine and 10 ml of water. After 12 hours, the solvent is distilled off and the residue is dissolved in ethyl acetate. The ethyl acetate layer is washed with 5% (W/V) NaHCO₃ and INHCl, followed by drying. The ethyl acetate is distilled off and the oily residue is treated with petroleum benzene. The crude crystals thus obtained are recrystallized from ethyl acetate-petroleum benzene. Yield: 8.2 g (63.3 mole%); melting point: 48.0-49.0°C; optical rotation: $[\alpha]_D^{21} -28.1^\circ$ (c=0.68, in DMF).

Elemental analysis, for $C_{14}H_{18}O_3N_2$:

Calcd. C, 64.10; H, 6.92; N, 10.68

Found C, 64.03; H, 6.92; N, 10.65

(b) *Synthesis of BOC-His-Pro-NHCH₃*

5.2 g (0.02 M) of Z-Pro-NHCH₃ are catalytically reduced in a conventional manner to H-Pro-NHCH₃. Separately, 11.8 g (0.02 M) of BOC-His(Tos)-OH.DCHA salt are suspended in ethyl acetate and vigorously shaken with 110 ml of ice-cooled 0.2N H₂SO₄. The ethyl acetate layer is washed twice with water and, after drying, the ethyl acetate is distilled off under reduced pressure. The oily residue and the H-Pro-NHCH₃ prepared above are dissolved together in 200 ml of a mixture of acetonitrile and methylene chloride (1:1 by volume), followed by ice-cooling. To this solution are added 4.12 g (0.02 M) of DCC and the mixture is stirred for 12 hours. The dicyclohexylurea by-product is filtered off and the filtrate is concentrated under reduced pressure. The oily residue is dissolved in 20 ml of N,N-dimethylformamide, followed by the addition of 2.70 g (0.04 M) of Hobt. The mixture is allowed to stand at room temperature for 5 hours. The solvent is distilled off *in vacuo* and the residue is dissolved in ethyl acetate. The ethyl acetate layer is extracted three times with water. The water layer is adjusted to pH 8 with NaHCO₃, saturated with sodium chloride and extracted with ethyl acetate. The ethyl acetate layer is washed twice with a saturated aqueous solution of sodium chloride and dried over anhydrous Na₂SO₄. The ethyl acetate is distilled off under reduced pressure and the oily residue is treated with petroleum benzene. The resulting powder is recovered by filtration. Yield: 2.60 g. (35.6 mole%); melting point: 75.0-80.0°C; optical rotation: $[\alpha]_D^{21} -22.7^\circ$ (c=0.74; in N,N-dimethylformamide).

Elemental analysis, for $C_{17}H_{27}O_4N_5$:

Calcd. C, 55.87; H, 7.45; N, 19.17

Found C, 56.16; H, 7.55; N, 18.39

(c) *Synthesis of Oct-His-Pro-NHCH₃*

In 15 ml of 25% (W/V) HBr/acetic acid are dissolved 1.46 g (0.004 M) of BOC-His-Pro-NHCH₃ and the solution is allowed to stand at room temperature for 20 minutes. Following addition of 150 ml of diethyl ether, the resulting precipitate is recovered by filtration and dried in a desiccator with NaOH for 12 hours. The dry powder thus obtained is dissolved in 15 ml of N,N-dimethylformamide and, under cooling, 1.12 ml (0.008 M) of triethylamine are added. The resulting salt is filtered off and the filtrate is used unchanged for the next reaction. Separately, 645 mg (0.004 M) of 3-oxo-5-carboxyperhydro-1,4-thiazine and 720 mg (0.004 M) of HONB are dissolved in 5 ml of N,N-dimethylformamide and, under cooling, 825 mg (0.004 M) of DCC are added. The mixture is stirred for 3 hours.

To this solution is added the amine solution previously prepared as above and the mixture is stirred at 4°C for 12 hours. The by-product dicyclohexylurea is filtered off and the filtrate is concentrated *in vacuo*.

The residue is treated with diethyl ether to obtain a powder which is then dissolved in 100 ml of a mixture of methanol and chloroform (1:9 by volume). The solution is run onto a column (5.0 × 12.0 cm) of silica gel. After the column is washed with 500 ml of the same

solvent, elution is carried out with a mixture of methanol and chloroform (4:6 by volume). The fractions rich in the contemplated compound are pooled and concentrated to remove the solvent. The residue is dissolved in a small amount of 0.1N acetic acid and further purified by passage through a column (5.5 × 40.0 cm) of Sephadex (Trade Mark) LH-20. The fractions rich in the desired compound are pooled and lyophilized. Yield: 1.04 g; optical rotation: $[\alpha]_D^{21} - 62.6^\circ$ ($c=0.63$, in H_2O); thin layer chromatography: $R_f=0.36$

Elemental analysis, for $C_{17}H_{24}O_4N_6S \cdot CH_3COOH \cdot 4H_2O$

Calcd. C, 42.21; H, 6.71; N, 15.55; S, 5.93

Found C, 41.88; H, 5.20; N, 15.63; S, 6.42

EXAMPLE 4

Synthesis of Oct-His-Pro-NHCH₂CH₂CH₂CH₃

(a) *Synthesis of Z-Pro-NHCH₂CH₂CH₂CH₃*

12.5 g (0.05 M) of Z-Pro-OH are converted to the active ester as in Example 3(a) and, after the addition of 5.7 ml. (0.06 M) of *n*-butylamine, the resulting mixture is stirred for 12 hours. The dioxane is distilled off under reduced pressure and the residue is dissolved in ethyl acetate. The ethyl acetate layer is washed with 5% (W/V) NaHCO₃ and 1N HCl, followed by drying. The ethyl acetate is distilled off under reduced pressure and, following the addition of petroleum benzine, the residue is filtered. The crude product is recrystallized from ethyl acetate. Yield: 14.0 g. (92.7 mole %); melting point: 94.0-95.0°C, optical rotation $[\alpha]_D^{21} - 39.6^\circ$ ($c=0.54$, in N,N-dimethylformamide)

Elemental analysis for $C_{17}H_{24}O_3N_2$

Calcd. C, 67.08; H, 7.95; N, 9.20

Found C, 66.83; H, 7.90; N, 9.05

(b) *Synthesis of BOC-His-Pro-NHCH₂CH₂CH₂CH₃*

Using 6.1 g (0.002 M) of Z-Pro-NHCH₂CH₂CH₂CH₃, the title compound is synthesized in the same manner as described in Example 3(b) for the synthesis of the methylamide. Yield: 4.4 g. (53.5 mole %), melting point: 65.0-70.0°C, optical rotation: $[\alpha]_D^{21} - 27.3^\circ$ ($c=0.70$, in N,N-dimethylformamide).

Elemental analysis, for $C_{20}H_{33}O_4N_5$

Calcd. C, 58.94; H, 8.16; N, 17.19

Found C, 59.05; H, 8.51; N, 16.47

(c) *Synthesis of Oct-His-Pro-NHCH₂CH₂CH₂CH₃*

Using 1.63 g (0.004 M) of BOC-His-Pro-NHCH₂CH₂CH₂CH₃, the title compound is synthesized in the same manner as described in Example 3(c) for the synthesis of the methylamide. Yield: 1.16 g.; optical rotation: $[\alpha]_D^{21} - 65.0^\circ$ ($c=0.60$, in water); thin-layer chromatography: $R_f=0.62$.

Elemental analysis, for $C_{20}H_{30}O_4N_6S \cdot CH_3COOH \cdot 4H_2O$

Calcd. C, 45.35; H, 7.25; N, 14.42; S, 5.50

Found C, 44.85; H, 5.75; N, 14.94; S, 6.36

EXAMPLE 5

Synthesis of Oct-His-Pro-NHCH₂CH₂C₆H₅

(a) *Synthesis of Z-Pro-NHCH₂CH₂C₆H₅*

Using 12.5 g. (0.05 M) of Z-Pro-OH, the above compound is synthesized in the same manner as described in Example 4(a) for the *n*-butyl-amide. Yield: 16.0 g. (90.9 mole %); melting point: 86.0-88.0°C; optical rotation: $[\alpha]_D^{21} - 39.6^\circ$ ($c=0.54$; in N,N-dimethylformamide).

Elemental analysis, for $C_{21}H_{24}O_3N_2$

Calcd. C, 71.57; H, 6.86; N, 7.59

Found C, 71.38; H, 6.72; N, 7.87

(b) *Synthesis of BOC-His-Pro-NHCH₂CH₂C₆H₅*

Using 7.0 g. (0.02 M) of Z-Pro-NHCH₂CH₂C₆H₅, the above compound is synthesized in the same manner as described in Example 3(a) for the production of the methylamide. Yield: 6.5 g. (71.4 mole %); melting point: 70.0-75.0°C (decomp.); optical rotation: $[\alpha]_D^{21} - 21.7^\circ$ ($c=0.75$, in N,N-dimethylformamide)

Elemental analysis, for $C_{24}H_{33}O_4N_5$:

Calcd. C, 63.27; H, 7.30; N, 15.38

Found C, 63.41; H, 7.49; N, 14.59

5 (c) *Synthesis of Oct-His-Pro-NHCH₂CH₂C₆H₅* 5

Using 1.82 g (0.004 M) of BOC-His-Pro-NHCH₂CH₂C₆H₅, the above compound is synthesized in the same manner as described in Example 3(c) for the synthesis of the methylamide. Yield: 1.37 g; optical rotation: $[\alpha]_D^{21} -80.5^\circ$ ($c=0.56$, in water); thin-layer chromatography:

10 $R_f=0.64$. 10

Elemental analysis, for $C_{24}H_{30}O_4N_6S \cdot CH_3COOH \cdot 3H_2O$:

Calcd. C, 52.52; H, 6.43; N, 14.13; S, 5.39

Found C, 52.15; H, 5.73; N, 14.32; S, 5.61

15 EXAMPLE 6 15

Synthesis of Cbl-His-Pro-NH₂

(a) *Synthesis of γ -carboxy- γ -butyrolactone*

20 In 200 ml. of water are suspended 29.4 g (0.2 M) of H-Glu-OH, and a solution of 16.8 g (0.24 M) of sodium nitrite in 120 ml. of water and 120 ml. of 2NH₂SO₄ are simultaneously added dropwise at room temperature over a period of about 90 minutes. The reaction mixture is allowed to stand for 12 hours. The water is distilled off under reduced pressure and hot acetone is added to the residue. The acetone is distilled off from the extract and the oily residue is purified by distillation under reduced pressure. The desired compound is obtained as a distillate between 170.0 and 175.0°C/0.5 mmHg. Yield: 14.3 g (55.0 mole %); 25 melting point: 50°C; optical rotation: $[\alpha]_D^{27} -11.4^\circ$ ($c=0.77$, in 2N NaOH). 25

Elemental analysis, for $C_5H_6O_4 \cdot 1/2H_2O$:

30 Calcd. C, 44.61; H, 4.87; N, 0.00

Found C, 44.69; H, 4.77; N, 0.00 30

(b) *Synthesis of Cbl-His-Pro-NH₂*

35 1.43 g. (0.011 M) of γ -carboxy- γ -butyrolactone and 3.46 g (0.013 M) of pentachlorophenol are dissolved in N,N-dimethylformamide and the solution is cooled with ice. Then, after the addition of 2.37 g. (0.0115 M) of DCC the solution is stirred for 4 hours. Separately, 3.85 g. (0.01 M) of Z-His-Pro-NH₂ are treated as in Example 1(c) to yield a solution of H-His-Pro-NH₂ in N,N-dimethylformamide-formamide. The above two solutions are combined and stirred at 4°C for 48 hours. The by-product dicyclohexylurea-urea is filtered off and the solvent is distilled off *in vacuo*. The residue is triturated with ethyl acetate and the resulting powder is recovered by filtration. The powder is washed twice with hot acetonitrile and, then, with ethyl acetate. The resulting powder is dissolved in water and the insoluble material is filtered off. The filtrate is freeze-dried. Yield: 3.80 g.; optical rotation: $[\alpha]_D^{22} -53.4^\circ$ ($c=0.56$, in water); thin-layer chromatography: $R_f=0.43$. 40

45 Elemental analysis, for $C_{16}H_{21}O_5N_5 \cdot 5.5 H_2O$: 45

Calcd. C, 41.56; H, 7.17; N, 15.15

Found C, 41.55; H, 5.41; N, 15.02

50 EXAMPLE 7 50

Synthesis of Cbl-His-Pip-NH₂

390 mg. (0.003 M) of γ -carboxy- γ -butyrolactone are treated in the same manner as in Example 6(b) to yield the pentachlorophenyl ester.

55 Separately, 1.20 g. (0.003 M) of Z-His-Pip-NH₂ are treated in the same manner as in Example 2 (c) to prepare a solution of H-His-Pip-NH₂ in N,N-dimethylformamide. The two solutions are combined and the mixture is stirred at 4°C for 12 hours. The by-product dicyclohexylurea is filtered off and the filtrate is distilled under reduced pressure. The residue is triturated with ethyl acetate and the resulting powder is washed twice with hot acetonitrile and dissolved in 0.1N acetic acid. The insoluble material is filtered off and the filtrate is purified by passage through a column (5.5 × 40.0 cm) of Sephadex (Trade Mark) LH-20. The fractions rich in the desired compound are pooled and lyophilized. 60

Yield: 400 mg.

optical rotation: $[\alpha]_D^{21} -49.1^\circ$ ($c=0.86$, in H₂O)

thin-layer chromatography: $R_f=0.45$

Elemental analysis, for $C_{17}H_{23}O_5N_5 \cdot 1/2CH_3COOH \cdot 3H_2O$

Calcd. C, 46.85; H, 6.77; N, 15.18

Found C, 46.62; H, 5.68; N, 15.35

5

EXAMPLE 8

Synthesis of Cbl-His-Pro-NHCH₃

By the procedure described in Example 6(b), 520 mg. (0.004 M) of γ -carboxy- γ -butyrolactone are converted to the pentachlorophenyl ester. Separately, 1.46 g. (0.004 M) of BOC-His-Pro-NHCH₃ are treated by the procedure described in Example 3 (c) to prepare a solution of H-His-Pro-NHCH₃ in N,N-dimethylformamide.

The two solutions are pooled and stirred at 4°C. for 12 hours. The by-product dicyclohexylurea is filtered off and the filtrate is distilled *in vacuo*. The residue is triturated with ethyl acetate. The resulting powder is reprecipitated twice from acetonitrile and ethyl acetate and dissolved in a small amount of 0.1N-acetic acid. The insolubles are filtered off. The filtrate is purified by passage through a column (5.5 $\times$ 40.0 cm) of Sephadex LH-20. The dominant fractions are pooled and lyophilized. Yield: 860 mg.; optical rotation: $[\alpha]_D^{21} -68.0^\circ$ (c=0.63; in H₂O); thin-layer chromatography: R_f=0.35.

20 Elemental analysis, for $C_{17}H_{23}O_5N_5 \cdot 1/2CH_3COOH \cdot 3H_2O$ 20

Calcd. C, 46.85; H, 6.77; N, 15.18

Found C, 46.88; H, 5.79; N, 15.17

EXAMPLE 9

25 *Synthesis of Cbl-His-Pro-NHCH₂CH₂CH₂CH₃* 25

Using 520 mg. (0.004 M) of γ -carboxy- γ -butyrolactone and 1.63 g. (0.004 M) of BOC-His-Pro-NHCH₂CH₂CH₂CH₃, the above compound is synthesized in the same manner as set forth in Example 8 for the methylamide. Yield: 852 mg.; optical rotation: $[\alpha]_D^{21} -66.4^\circ$ (c=0.67, in H₂O) thin-layer chromatography: R_f=0.60

30 30

Elemental analysis, for $C_{20}H_{29}O_5N_5 \cdot 1/2CH_3COOH \cdot 3.5 H_2O$

Calcd. C, 49.21; H, 7.47; N, 13.67

Found C, 48.85; H, 6.40; N, 13.80

35 EXAMPLE 10 35

Synthesis of Cbl-His-Pro-NHCH₂CH₂C₆H₅

Using 520 mg. (0.004 M) of γ -carboxy- γ -butyrolactone and 1.82 g. (0.004 M) of BOC-His-Pro-NHCH₂CH₂C₆H₅, the above compound is synthesized in the same manner as Example 8 for the synthesis of the methylamide. Yield: 517 mg. optical rotation: $[\alpha]_D^{21} -78.8^\circ$ (c=0.52, in H₂O) thin-layer chromatography: R_f=0.63

40 40

Elemental analysis, for $C_{24}H_{29}O_5N_5 \cdot 1/2CH_3COOH \cdot 6H_2O$

Calcd. C, 49.58; H, 7.16; N, 11.57

Found C, 49.63; H, 5.43; N, 11.96

45 45

EXAMPLE 11

Synthesis of Oct-His-Pro-NHCH₂CH₂CH₂CH₂CH₃

(a) *Synthesis of Z-His-Pro-O^tBu*

Z-Pro-O^tBu 10.0 g. (0.033 M) in methanol is hydrogenated over a Pd catalyst. The catalyst is removed by filtration and the filtrate is condensed under reduced pressure. To the residue is added a cold solution of Z-His-N₃ [prepared from Z-His-NHNH₂ 9.1 g. (0.03 M)] in ethyl acetate. The mixture is stirred at 4°C for 72 hours and then washed with a 5% (W/V) aqueous solution of sodium bicarbonate and water. The solvent is evaporated under reduced pressure. The residue is triturated with petroleum ether and recrystallized from ethyl acetate. Yield: 9.5 g. (71.4 mole %); melting point: 143-145°C; optical rotation: $[\alpha]_D^{26} -41.7^\circ$ (c=0.47, in DMF).

55 55

Elemental analysis, for $C_{23}H_{30}O_5N_4$:

Calcd. C, 62.42; H, 6.83; N, 12.66

60 Found C, 62.15; H, 6.75; N, 12.71 60

(b) *Synthesis of Oct-His-Pro-OH*

DCC 2.06 g. (0.01 M) is added to a solution of H-His-Pro-O^tBu prepared from Z-His-Pro-O^tBu 4.42 g. (0.01 M) by catalytic hydrogenation, 3-oxo-5-carboxyperhydro-1,4-thiazine 1.61 g. (0.01 M) and HONB 1.79 g. (0.01 M) in DMF and the mixture is stirred at

65 65

28

room temperature for 12 hours. The urea formed is filtered off and the filtrate is evaporated *in vacuo*. The residue is triturated with diethyl ether, then collected by filtration and dried over P_2O_5 *in vacuo*. The dried powder is treated with 25 ml. of trifluoroacetic acid at room temperature for 3 hours to remove the *t*-butyl ester. The excess of acid is evaporated under reduced pressure and the residue is triturated with diethyl ether, then collected by filtration and dried over NaOH pellets *in vacuo*.

The dried powder is dissolved in a mixture of chloroform-methanol (9:1 by volume) and charged on the top of a silica gel column (5.0 × 8.0 cm). The column is washed with the same solvent and then eluted with a mixture of chloroform-methanol (6:4 by volume). The main fractions are collected and concentrated under reduced pressure. The residue is dissolved in 50 ml. of 0.2N-HCl under ice-cooling and then lyophilized. Yield: 2.60 g. (60.5 mole %); optical rotation: $[\alpha]_D^{21} - 61.9^\circ$ ($c=0.49$, in water); thin-layer chromatography: $R_f=0.35$.

Elemental analysis, for $C_{16}H_{21}O_5N_5S \cdot HCl \cdot 2.5H_2O$ -
Calcd. C, 40.29; H, 5.72; N, 14.68; S, 6.23
Found C, 40.28; H, 5.09; N, 14.65; S, 6.19

(c) *Synthesis of Oct-His-Pro-NHCH₂CH₂CH₂CH₃*

DCC 620 mg. (0.003 M) is added to a mixture of Oct-His-Pro-OH 432 mg. (0.001 M), HONB 269 mg. (0.0015 M) and *n*-amylamine 0.29 ml. (0.0025 M) in DMF and the mixture is stirred at room temperature for 48 hours. The urea formed is filtered off and the filtrate is evaporated *in vacuo*. The residue is triturated with diethyl ether and the resulting powder is purified in the same manner as described in Example 3(c). Yield: 110 mg.; optical rotation: $[\alpha]_D^{21} - 65.4^\circ$ ($c=0.35$, in water); thin-layer chromatography: $R_f=0.73$.

Elemental analysis, for $C_{21}H_{32}O_4N_6S \cdot CH_3COOH \cdot 2H_2O$
Calcd. C, 49.27; H, 7.19; N, 14.99; S, 5.72
Found C, 48.83; H, 6.56; N, 15.36; S, 5.57

EXAMPLE 12

The compounds listed below are prepared in the same manner as described in Example 11 (c) by using the corresponding amines.

Synthesis of Oct-His-Pro-NHCH₂CH₃

Yield 137 mg.; optical rotation: $[\alpha]_D^{21} - 67.7^\circ$ ($c=0.31$, in water); thin-layer chromatography: $R_f=0.55$;

Elemental analysis, for $C_{18}H_{26}O_4N_6S \cdot CH_3COOH \cdot H_2O$:
Calcd. C, 47.99; H, 6.44; N, 16.79; S, 6.41
Found C, 47.74; H, 6.43; N, 16.74; S, 6.15

Synthesis of Oct-His-Pro-NHCH₂CH₂CH₃

Yield: 90 mg.; optical rotation: $[\alpha]_D^{21} - 51.6^\circ$ ($c=0.31$, in water); thin-layer chromatography: $R_f=0.65$

Elemental analysis, for $C_{19}H_{28}O_4N_6S \cdot CH_3COOH \cdot 2H_2O$:
Calcd. C, 47.36; H, 6.81; N, 15.78; S, 6.02
Found C, 47.29; H, 6.13; N, 15.49; S, 6.02

Synthesis of Oct-His-Pro-NHCH₂CH₂CH₂ACH₃

Yield: 105 mg.; optical rotation: $[\beta]_D^{21} - 66.4^\circ$ ($c=0.54$, in water); thin-layer chromatography: $R_f=0.70$

Elemental analysis, for $C_{22}H_{34}O_4N_6S \cdot CH_3COOH \cdot 2H_2O$:
Calcd. C, 50.16; H, 7.36; N, 14.62; S, 5.58
Found C, 49.76; H, 7.18; N, 15.78; S, 5.66

By the same procedure as above, the following peptides can be produced.

Oct-His-Pro-NHCH₂CH₂OCH₃,
 Cbl-His-Pro-NHCH₂CH₂OCH₃,
 Oct-His-Pro-NHCH₂CH₂OH,
 Cbl-His-Pro-NHCH₂CH₂OH,
 Oct-His-Tac-NH₂,
 Oct-His-Tac-NHCH₂CH₂CH₂CH₃,
 Oct-His-Tac-NHCH₂CH₂OCH₃,
 Oct-His-Tac-NHCH₂CH₂OH,
 Cbl-His-Tac-NH₂,
 Cbl-His-Tac-NHCH₂CH₂CH₂CH₃,
 Cbl-His-Tac-NHCH₂CH₂OCH₃,
 Cbl-His-Tac-NHCH₂CH₂OH,

Pharmacological test results

Test procedure

(1) TSH-releasing activity

Using rats, a test was performed in accordance with the method of Schally *et al.* [J. Biol. Chem. 244, 4077, (1969)].

(2) Anaesthesia antagonism (anti-depressant activity)

55 mg/kg of pentobarbital sodium were intraperitoneally given to mice (ICR/JCL, male, 4 weeks old) and, when the righting reflex had disappeared, namely after 10 minutes, the compound (I) and TRH (physiological saline for control) were intravenously administered, and the times which had elapsed before a recovery of the righting reflex took place [Prange *et al.*, Life Sci 14, 447-55, (1974)] were measured.

(3) Activity-increasing action

Using groups of 10 mice (ICR/JCL, male, 4 weeks old), each mouse was placed in an activity wheel and the activity-increasing action of the medication on the mouse was observed. The cumulative number of rotations caused by the spontaneous activity of the mice during a period of 3 hours following the intravenous administration of compound (I) was compared with the number of revolutions for the control group.

(4) Dopamine activity-potentiating effect

Using groups of 10 male mice (ICR/JCL) with one of its caudate nuclei destroyed by suction the effects of compound (I) upon the number of turns and the percentage incidence of head turning as caused by 0.25 mg/kg of apomorphine (i.p.), i.e. a dopamine-receptor stimulant, under the influence of premedication (18-22 hours) with 2 mg/kg of reserpine (i.p.) were examined.

Thus, compound (I) was given 30 minutes before the administration of apomorphine and the result was compared with that for the untreated (control) group. This test was performed to ascertain the potentiating effect of the compound upon the central nervous system action of dopamine, the principle of the test being the same as that of the experiment performed by Everett *et al.* [Fed. Proc. 23, 198, (1964)] for testing the DOPA synergistic effect which involved the administration of DL-DOPA.

The relative effects of compound (I) by the above 4 procedures were expressed in terms of the ratios of effect of the same dose of compound (I) as that of TRH with the effects of TRH being taken as unity. The results are summarized in the following table.

TABLE 1

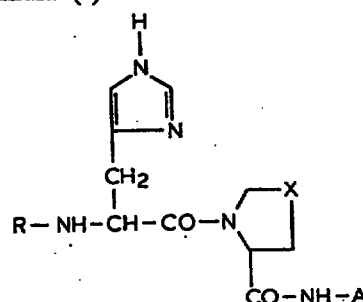
Effect	TSH-releasing effect	Anaesthesia antagonism	Spontaneous activity-increasing effect	Dopamine-potentiating effect
Compound				
TRH	1	1	1	1
Oct-His-Pip-NH ₂	0.5	1.57	5	4.00
Cbl-His-Pro-NH ₂	0.01-0.02	1.25	1.0	16.00

Other compounds (I) according to this invention can be produced by the same method as

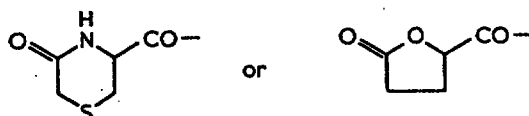
described in the foregoing examples and tested for their pharmacological effects by the same testing procedures as hereinbefore described.

WHAT WE CLAIM IS:-

1. Compounds of the formula (I):



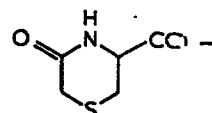
wherein A is hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy; R is



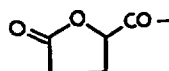
and X is -S- or $-(CH_2)_n$ - where n is 1 or 2, and R and other constituent amino acid residues may each be of L-, D- or DL- configuration, and pharmacologically acceptable salts thereof with inorganic acids or organic acids.

2. A compound or salt as claimed in Claim 1, wherein R and other constituent amino acid residues are in the L-configuration.

3. A compound or salt as claimed in Claim 1 or 2, wherein R is



4. A compound or salt as claimed in Claim 1 or 2, wherein R is



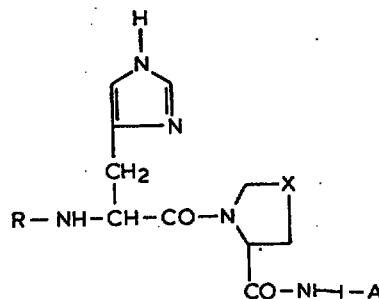
5. A compound of the formula (I) as defined in Claim 1 or a pharmaceutically acceptable salt thereof with an inorganic or organic acid substantially as herein described with reference to any of the specific examples.

6. L-2-oxo-tetrahydrofuran-5-carbonyl-L-histidyl-L-prolineamide.

7. L-3-oxo-perhydro-1,4-thiazine-5-carbonyl-L-histidyl-L-proline n-butylamide.

8. A pharmaceutically acceptable salt of a compound as claimed in claim 6 or 7 with an inorganic or organic acid.

9. A process for producing a compound of the formula (I):





15

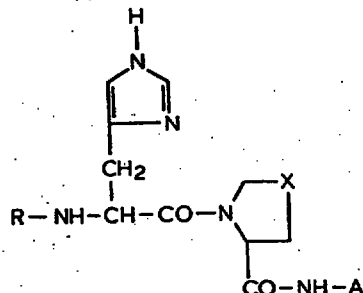
1 579 264

15

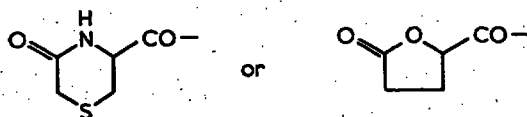
described in the foregoing examples and tested for their pharmacological effects by the same testing procedures as hereinbefore described.

WHAT WE CLAIM IS:-

1. Compounds of the formula (I):



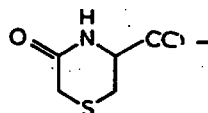
wherein A is hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy; R is



and X is -S- or $-(CH_2)_n-$ where n is 1 or 2, and R and other constituent amino acid residues may each be of L-, D- or DL- configuration, and pharmacologically acceptable salts thereof with inorganic acids or organic acids.

2. A compound or salt as claimed in Claim 1, wherein R and other constituent amino acid residues are in the L-configuration.

3. A compound or salt as claimed in Claim 1 or 2, wherein R is



4. A compound or salt as claimed in Claim 1 or 2, wherein R is



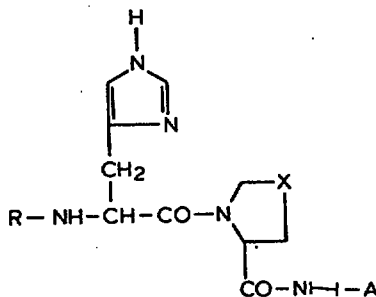
5. A compound of the formula (I) as defined in Claim 1 or a pharmaceutically acceptable salt thereof with an inorganic or organic acid substantially as herein described with reference to any of the specific examples.

6. L-2-oxo-tetrahydrofuran-5-carbonyl-L-histidyl-L-prolineamide.

7. L-3-oxo-perhydro-1,4-thiazine-5-carbonyl-L-histidyl-L-proline n-butylamide.

8. A pharmaceutically acceptable salt of a compound as claimed in claim 6 or 7 with an inorganic or organic acid.

9. A process for producing a compound of the formula (I):



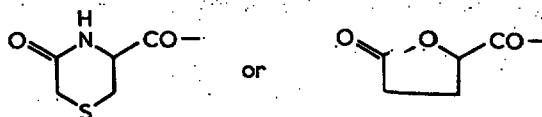


16

1 579 264

16

wherein A is hydrogen, alkyl, aralkyl, alkoxyalkyl, hydroxyalkyl or alkoxy; R is



and X is -S- or $-(CH_2)_n-$ where n is 1 or 2, and R and other constituent amino acid residues may each be of L, D- or DL- configuration, or a pharmacologically acceptable salt thereof with an inorganic or organic acid, in which an amino acid or a peptide fragment constituting one component of the compound (I) is reacted with the fragment constituting the remaining component of the compound (I), any group or groups not involved in amide bond formation being protected by flocking if necessary and later unblocked if necessary, and in any group or groups which take part in said formation being activated if necessary, and, if desired the resulting compound (I) is reacted with an inorganic acid or an organic acid yielding therewith the desired pharmacologically acceptable salt.

10. A process according to claim 9 substantially as herein described with reference to any of the specific examples.

11. Compounds (I) as defined in Claim 9, or pharmacologically acceptable salts thereof, when produced by a process according to Claim 9 or 10.

12. A pharmaceutical composition comprising a compound (I) or pharmaceutically acceptable salt thereof with an inorganic acid or organic acid as claimed in any of Claims 1 to 8, or in Claim 11, together with a pharmaceutically acceptable carrier or diluent therefor.

ELKINGTON AND FIFE,
Chartered Patent Agents,
High Holborn House,
52/54 High Holborn,
London WC1V 6SH.
Agents for the Applicants.