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PATENT SPECIFICATION

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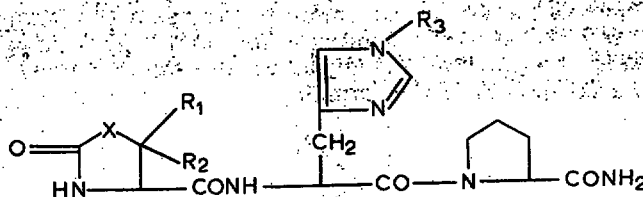


(54) TRIPEPTIDES

(71) We, TAKEDA YAKUHH KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES, LTD., of 27, Doshomachi 2-chome, Higashi-ku, Osaka, Japan, a joint stock company organised under the laws of 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:—

The invention relates to novel tripeptides and to a process for their preparation.

Thus, the invention provides tripeptides of the formula



wherein each of R_1 , R_2 and R_3 is hydrogen or an alkyl group having 1 to 3 carbon atoms; and X is oxygen or sulfur, and pharmaceutically acceptable acid salts thereof.

In the present specification and the claims, amino acids and peptides may be shown by the abbreviations which have been accepted in this field or have been adopted by the IUPAC-IUB (International Union of Pure and Applied Chemistry-International Union of Biochemistry) Commission on Biological Nomenclature. Abbreviations used throughout the specification are exemplified as below:

Ser: L-Serine
 His: L-Histidine
 Pro: L-Proline
 Thr: L-Threonine
 Cys: L-Cysteine

Ser: L-2-oxo-oxazolidine-4-carboxylic acid

Thr: L-Trans-5-methyl-2-oxo-oxazolidine-4-carboxylic acid

Cys: L-2-oxo-thiazolidine-4-carboxylic acid

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DDC: N,N'-dicyclohexylcarbodiimide

Z: Benzyloxycarbonyl

BzL: Benzyl

His (im-3-Me): Imidazole-3-methyl-L-histidine

IBOC: Isobornylcarbonyl

O

—NHNH—C(=O)tBu: t-Butyloxycarbonyl-hydrazide

HONB: N-Hydroxy-5-norbornene-2,3-dicarboximide

HOSu: N-Hydroxysuccinimide

HOBT: N-Hydroxybenztriazole

Me: Methyl

Referring to the above definitions, the alkyl group represented by the symbols R_1 , R_2 and R_3 is exemplified by methyl, ethyl or isopropyl.

In a particular embodiment of the invention there is provided a compound of general formula (I) or a pharmaceutically acceptable salt thereof in which R_1 is hydrogen and R_2 is an alkyl group having 1 to 3 carbon atoms.

In another embodiment there is provided a compound of general formula (I) or a pharmaceutically acceptable salt thereof in which R_3 is an alkyl group having 1 to 3 carbon atoms.

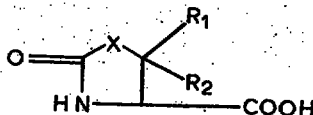
We have synthesized the compound (I) and found unexpectedly:

- (1) that the compound (I) exhibits a thyrotropin-releasing hormone action; and
- (2) that the compound (I) unlike known thyrotropin-releasing hormones does not exhibit a side-effect on the central nervous system such as Dopa (β -(3,4-Dihydroxyphenyl)-L-alanine) potentiating action at the dose level of producing the thyrotropin-releasing hormone action.

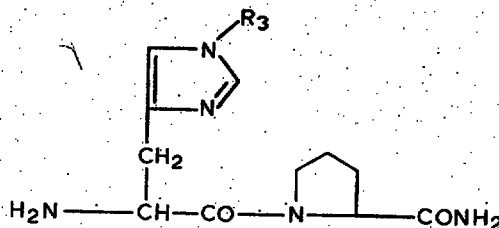
The present invention was based upon these findings.

It is an object of the present invention to provide a compound (I).

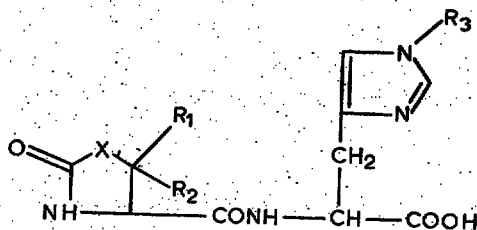
The invention also provides a process for producing a compound of the general formula (I) or a pharmaceutically acceptable salt thereof which comprises condensing (1) a compound of the formula



wherein R_1 , R_2 and X have the same meaning as defined above with a compound of the formula



wherein R_3 has the meaning as defined above or (2) a compound of the formula



wherein R_1 , R_2 , R_3 and X have the same meaning as defined above with a compound of the formula

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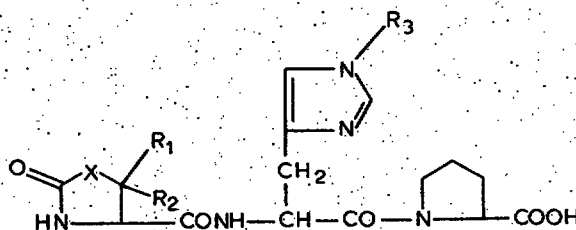
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or (3) a compound of the formula



wherein R_1 , R_2 , R_3 and X have the same meaning as defined above with NH_2 ; and, if desired reacting the resulting compound of general formula (I) with an acid to obtain a pharmaceutically acceptable salt of the compound (I).

Thus in the process according to the present invention an amino acid or a peptide fragment (hereinafter referred to as reagent (A)) is reacted with ammonia or an amino compound (hereinafter referred to as reagent (B)) to yield a compound of general formula (I).

Basic combinations of the reagents (A) and (B) are shown in the following Table 1.

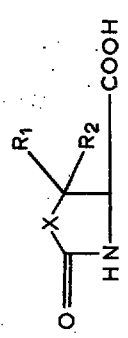
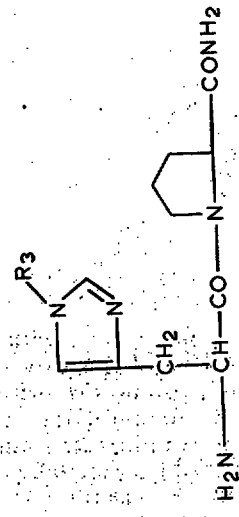
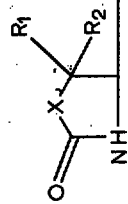
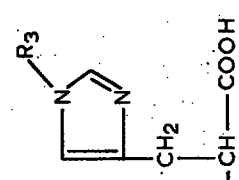
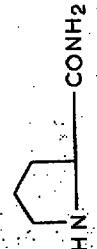
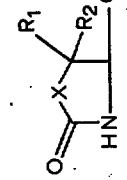
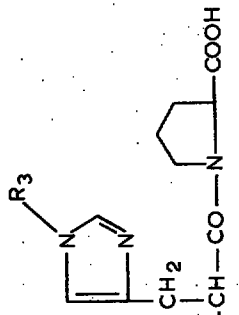
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TABLE I

Reagent Combination	A	B
1		
2	 	
3	 	

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The present condensation reaction can be conducted by employing suitably *per se* conventional condensation techniques for the formation of the peptide bonds. The condensation techniques are exemplified by the azide process, the chloride process, the acid anhydride process, the mixed acid anhydride process, the DCC process, the active ester process, the process employing Woodward's reagent K, the carbodiimidazole process and the oxidation-reduction process and DCC/HONB process. Those processes are described in the text-book: "The Peptides" Vol. I (1966) written by Schröder and Lubke and published by Academic Press, New York, U.S.A.

Prior to carrying out the present condensation reaction, the amino and/or carboxyl group or groups of the starting amino acids and/or peptides which are not involved in the intended peptide bond formation may be protected by a conventional protective group or conventional protective groups using conventional techniques. The carboxyl group of the starting amino acid or peptide may be protected, for instance, by the formation of metal salts (e.g. the sodium or potassium salt) or in the form of esters (e.g. the methyl, ethyl, benzyl, *p*-nitrobenzyl, *t*-butyl or *t*-amyl ester).

The protective group for the amino group of the starting amino acids or peptides may be, for example, formyl, phthalyl, trifluoroacetyl, *p*-toluenesulfonyl, benzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-chlorobenzyloxycarbonyl, *t*-butyloxycarbonyl, *t*-amyloxycarbonyl, trichloroacetyl, isobornyloxycarbonyl, diphenylmethyloxycarbonyl or triphenylmethyl.

The imidazole ring in the moiety R_2 wherein R_2 takes hydrogen may be protected prior to the present condensation reaction with a *per se* conventional protective group such as the benzyl, *p*-toluenesulfonyl, 2,4-dinitrophenyl, isobornyloxycarbonyl, *t*-butyloxycarbonyl or benzyloxycarbonyl group by *per se* conventional techniques.

The following are practicable procedures in accordance with the present process.

(i) Reagent (A) having a free carboxyl group as its C-terminal group and a protected amino group is reacted with reagent (B) having a free amino group as its N-terminal group and a protected carboxyl group in the presence of a dehydrating agent.

(ii) Reagent (A) having an activated carboxyl group as its C-terminal group and a protected amino group is reacted with reagent (B) having a free amino group as its N-terminal group, and

(iii) Reagent (A) having a free carboxyl group as its C-terminal group and a protected amino group is reacted with reagent (B) having an activated amino group as its N-terminal group and a protected carboxyl group.

In the present process there may suitably be employed conventional dehydrating agents, for example, the so-called carbodiimide reagents (e.g. dicyclohexylcarbodiimide).

An activated carboxyl group of the reagent (A) is exemplified by the corresponding acid anhydride, azide or active ester (e.g. the corresponding ester of the alcohol or phenol such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethylalcohol, *p*-nitrophenol, N-hydroxy-5-norbornene-2,3 dicarboximide, N-hydroxysuccinimide, N-hydroxyphthalimide or N-hydroxybenztriazole). Among those esters, the N-hydroxy-5-norbornene-2,3 dicarboximide ester is preferred.

An activated amino group of the reagent (B) includes the corresponding phosphorous acid amide.

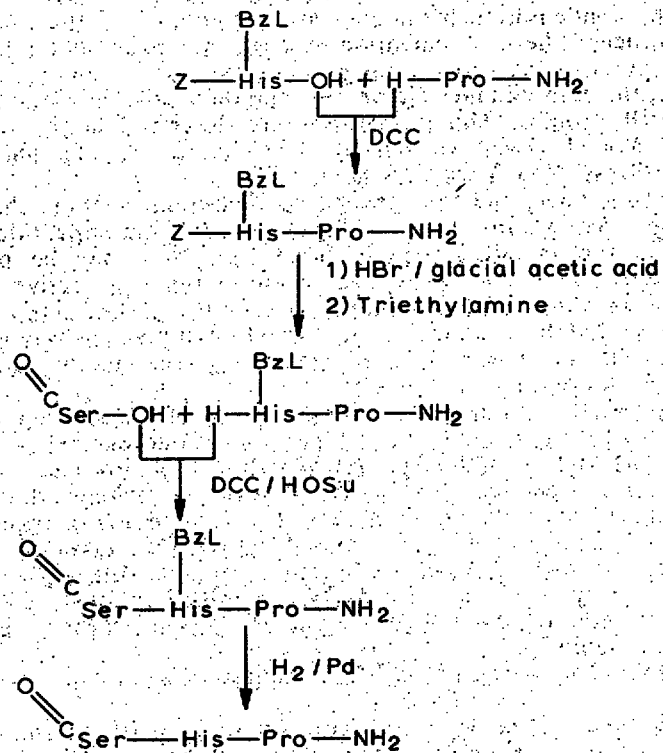
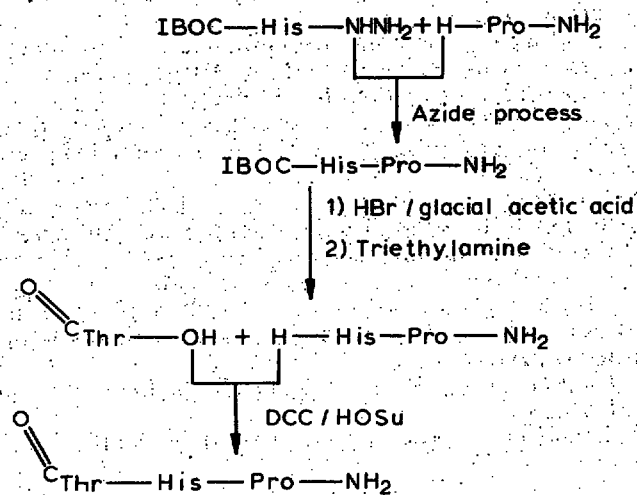
Some embodiments of the present process are shown in the following charts.

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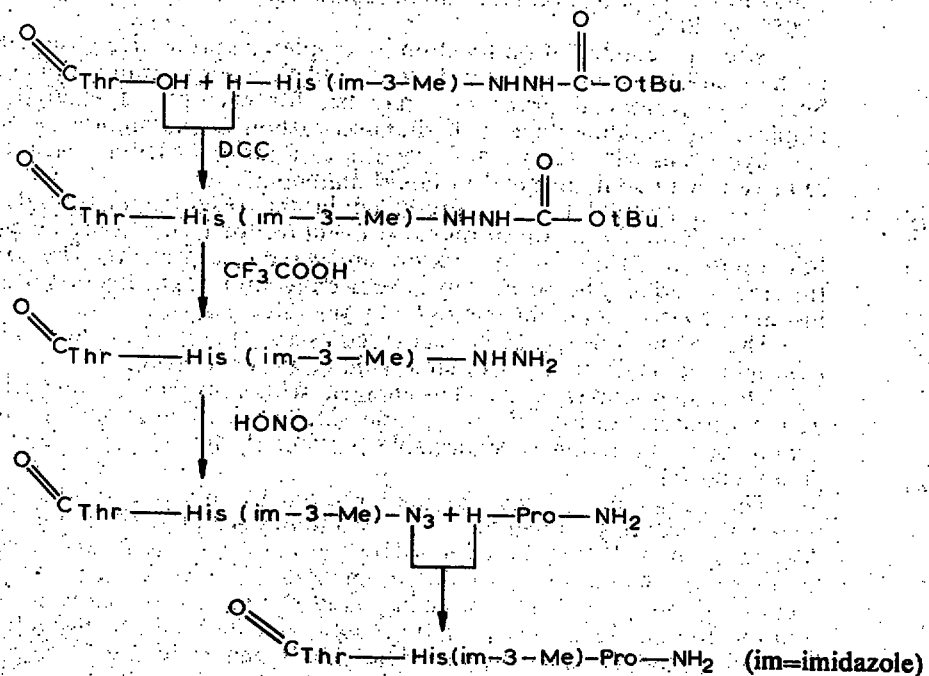
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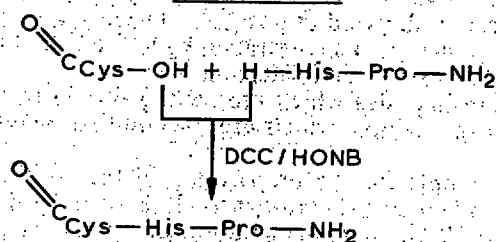
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Procedure 1Procedure 2

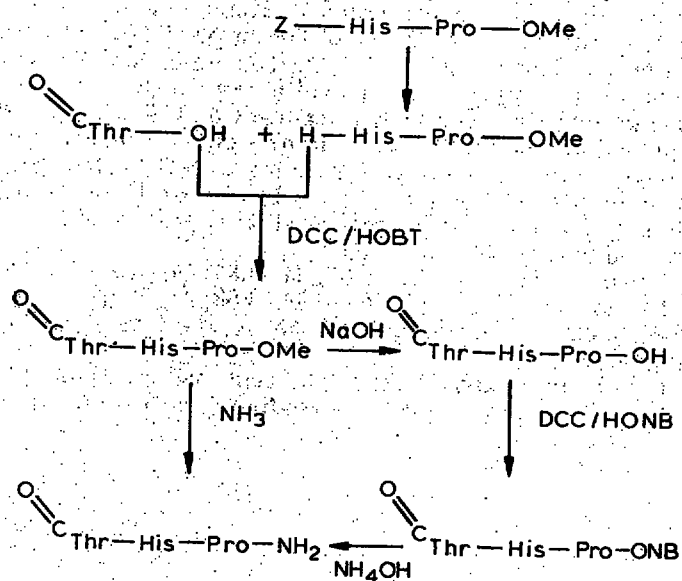
Procedure 3



Procedure 4



Procedure 5



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The reaction temperature is suitably chosen from the range employed in conventional peptide bond formation reactions and may be a temperature of from -20°C to 30°C .

After the condensation reaction is complete, a protective group, if it remains in the resulting compound, can be removed by a *per se* conventional procedure. Such a conventional procedure may be, for example, a catalytic reduction employing palladium-black, palladium-on-carbon or platinum as a catalyst; and acid hydrolysis with, for example, hydrogen fluoride or trifluoroacetic acid; or a reduction with sodium metal in liquid ammonia.

The compound (I) thus produced in the resulting mixture is recovered therefrom by a *per se* conventional procedure, for instance, by evaporating the liquid portion of the resulting mixture under reduced pressure. The compound (I) thus recovered may be purified by a *per se* conventional purification procedure, for instance, by a column chromatography on silica gel.

The compound (I) may be recovered as an acid salt of an inorganic acid (e.g. hydrochloric acid) or of an organic acid (e.g. acetic acid).

The compound (I) is useful as an agent with a thyrotropin-releasing hormone action without causing an undesirable side-effect such as a Dopa potentiating action. Such an action is remarkable particularly in the L configuration of the compound (I).

The compound (I) may be administered by injection or the oral route to mammals including human beings for promoting lactation or inhibiting gestation. The compound (I) may be administered in conventional dosage form such as a tablet, a capsule, powder, granules, or an injection solution which are prepared by a conventional procedure. The compound (I) may be prepared as an injection solution by dissolving 100 γ of the compound in 1 ml. of physiological saline. The compound (I) may be stored in the frozen state after being lyophilized together with mannitol and put into use after being dissolved in a physiological saline. The dose may be variable depending upon the individual compound (I), the symptoms of the disease, the kind of host, etc. The usual dose may range from 1 γ to 1 mg/kg. of body weight per administration by injection and from 10 γ to 100 mg. on the same basis upon oral administration.

Amino acids and peptides which are employable in the present process are prepared by known procedures which are exemplified below.

Reference Example for the preparation of the starting compounds

Preparation of L-2-oxo-oxazolidine-4-carboxylic acid

In 960 ml. of a 12.5% aqueous solution of potassium hydroxide, there are dissolved 27.3 g. (0.26 mole) of L-serine and under cooling at $4-5^{\circ}\text{C}$ and vigorous stirring, a cold solution of 58 g. of phosgene in 40 ml. of toluene is added dropwise over a period of 75 minutes. The mixture is stirred at $4-7^{\circ}\text{C}$ for one hour, at the end of which time the toluene layer is separated. The water layer is washed twice with 250 ml. of ether. The water layers are then rendered acid to pH 3 with 3N hydrochloric acid and the water is evaporated under reduced pressure. The residue is extracted with 800 ml. of hot methanol, the latter being then removed by evaporation. The residue is dissolved in water and the solution is run onto a column (3.5x38cm) of a strong acid ion-exchange resin (i.e. Dowex-50 (Trade Mark) produced by The Dow Chemical Co., U.S.A.). The effluent and washings are pooled and concentrated to dryness under reduced pressure. The residue is thoroughly dried and recrystallized from ethyl acetate petroleum benzin. The procedure gives 16.6 g. (48 %) of L-2-oxo-oxazolidine-4-carboxylic acid. Melting point: $112-113^{\circ}\text{C}$, $[\alpha]_D^{25} = -18.7^{\circ}$ ($c=1.0$, water). Elemental analysis for $\text{C}_4\text{H}_5\text{O}_4\text{N}$

Calcd. C, 36.65; H, 3.85; N, 10.69

Found C, 36.86; H, 3.68; N, 10.67

By a similar procedure to the above, the following starting compounds are prepared.

L-2-oxothiazolidine-4-carboxylic acid, melting point: $170-172^{\circ}\text{C}$.

L-trans-5-methyl-2-oxo-oxazolidine-4-carboxylic acid, melting point: $134-136^{\circ}\text{C}$.

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Example 1.

Preparation of L-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide

[Ser-His-Pro-NH₂]

In the presence of 0.1 ml. of anisole, there are dissolved 1.1 g. [2.55 millimoles (abbreviated as "mM")] of isobornyloxycarbonyl-L-histidyl-L-prolineamide in 10 ml. of trifluoroacetic acid and the reaction is allowed to proceed at room temperature for 40 minutes. Finally, 2 ml. of 4N HCl-dioxane are added, followed by evaporation. The residue is treated with ether and the resulting powder is collected by filtration and dried in a desiccator over sodium hydroxide. The procedure gives powder of L-histidyl-L-prolineamide dihydrochloride. The same compound can also be obtained by reducing carbobenzoxy-L-histidyl-L-prolineamide in ethanol and in the presence of two molecular equivalents of HCl using palladium black as a catalyst, removing the catalyst by filtration and concentrating the filtrate to dryness under reduced pressure. This powder together with 400 mg. (3.08 mM) of L-2-oxo-oxazolidine-4-carboxylic acid and 550 mg. (3.08 mM) of N-hydroxy-5-norbornene-2,3-dicarboximide, is dissolved in 5 ml. of dimethylformamide and, then, under cooling with ice, 0.67 ml. of N-ethylmorpholine is added. Thereafter, 635 mg. (3.08 mM) of N,N'-dicyclohexylcarbodiimide are added and the mixture is stirred for 12 hours. The dicyclohexylurea that has separated out is removed by filtration and the filtrate is concentrated to dryness under reduced pressure. The residue is dissolved in a mixture of chloroform and methanol (9:1) and the solution is run onto a column (4x8cm) of silica gel. The column is washed with a mixture of chloroform and methanol (7:3) and the washed product is eluted with 60 % aqueous methanol. The eluate is distilled under reduced pressure and the residue is dissolved in water and adsorbed on a column (2x30cm) of a weakly acid cation-exchanger (i.e. carboxymethyl-Sephadex (Trade Mark), a product of Pharmacia, A.S. Sweden). The column is then irrigated by the gradient elution method, using aqueous solutions of ammonium acetate of from 0.005 mole to 0.2 mole (500 ml./500 ml.). The desired product emerges in the fractions corresponding to 390 ml. to 550 ml. and, therefore, these fractions are collected and lyophilized. The procedure gives 523 mg. (56 %) white powder of the acetate of L-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

$[\alpha]_D^{25} = -45.3^\circ$ (c=0.53, acetic acid); amino acid analysis (5.7 N HCl, 105°C, 16 hours): Ser:His:Pro = 0.85:1.03:1.00.

The R_f value by thin layer chromatography [Solvent:n-butanol:ethyl acetate:acetic acid:water = 1:1:1:1. Plate: Woelm precoated TLC sheet silica gel F-254/366 (Woelm, Wt. Germany)]=0.18 Negative to ninhydrin reagent and positive to Pauly reagent.

Example 2.

Preparation of L-trans-5-methyl-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide

650 mg. (2 mM) of L-histidyl-L-prolineamide dihydrochloride, 311 mg. (2.2 mM) of L-trans-5-methyl-2-oxo-oxazolidine-4-carboxylic acid and 250 mg. of N-hydroxysuccinimide are dissolved in 5 ml. of dimethylformamide. Under cooling with ice, 0.51 ml. of N-ethylmorpholine and 453 mg. (2.2 mM) of N,N'-dicyclohexylcarbodiimide are added. The mixture is stirred for 15 hours. The reaction mixture is filtered to remove the insolubles and the filtrate is concentrated to dryness under reduced pressure. The residue is dissolved in a mixture of chloroform and methanol (9:1) and adsorbed on a column (3x7cm) of silica gel. The column is first washed with a mixture of chloroform and methanol (8:2) and the desired product is then eluted with a mixture of chloroform and methanol (4:6). The solvent is evaporated under reduced pressure and the residue is dissolved in 5 ml. of water. The aqueous solution is run onto a column (2x50cm) of agar gel (i.e. Sephadex (Trade Mark) LH-20, a product of Pharmacia, A.S. Sweden) and the fractions containing the desired product are lyophilized. The procedure gives 250 mg. (33 %) of white powder of L-trans-5-methyl-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

$[\alpha]_D^{25} = -41.1^\circ$ (c=0.52, 5 % acetic acid)

Amino acid analysis: Thr:His:Pro = 1.00:1.00:0.97.

Rf value by thin layer chromatography on the same type of the plate with the same kind of the solvent as in Example 1 = 0.24.

Example 3.

Preparation of L-2-oxothiazolidine-4-carbonyl-L-histidyl-L-prolineamide

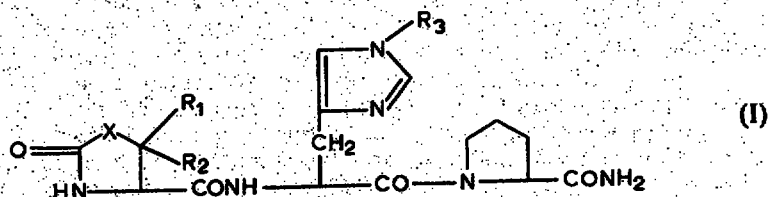
In 6 ml. of dimethylformamide, there are dissolved 260 mg. (2 mM) of L-2-oxothiazolidine-4-carboxylic acid and 650 mg. of L-histidyl-L-prolineamide dihydrochloride together with 271 mg. of N-hydroxybenztriazole. Under cooling with ice, 0.3 ml. of triethylamine and then 460 mg. of N,N'-dicyclohexylcarbodiimide are added. The mixture is stirred for 20 hours, after which time the reaction mixture is filtered. The filtrate is concentrated to dryness under reduced pressure and the concentrate is dissolved in 20 ml. of water. The insolubles, which are formed in minor amounts, are removed by filtration and the filtrate is adsorbed on a column (2x30cm) of carboxymethyl-Sephadex (Trade Mark). The column is then irrigated with aqueous solutions of ammonium acetate from water to 0.1 mole concentration (500 ml./500 ml.) by the gradient elution method. The fractions containing the contemplated product are pooled and lyophilized. The procedure gives 430 mg. of white powder.

Amino acid analysis: CysH:His:Pro = 0.73:1.00:0.98.

Rf value by thin layer chromatography on the same type of plate with the same kind of the solvent as in Example 1 = 0.20.

WHAT WE CLAIM IS:—

1. A compound of the formula



wherein each of R_1 , R_2 and R_3 is hydrogen or an alkyl group having 1 to 3 carbon atoms and X is oxygen or sulfur, or a pharmaceutically acceptable acid salt thereof.

2. A compound as claimed in Claim 1, wherein R_1 is hydrogen and R_2 is an alkyl group having 1 to 3 carbon atoms.

3. A compound as claimed in Claim 1, wherein R_3 is an alkyl group having 1 to 3 carbon atoms.

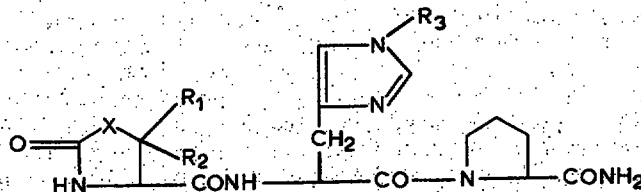
4. L-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

5. L-trans-5-methyl-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

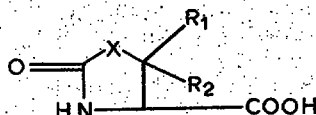
6. L-2-oxothiazolidine-4-carbonyl-L-histidyl-L-prolineamide.

7. A compound (I) as defined in claim 1 substantially as herein described with reference to any of the specific examples.

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



wherein each of R_1 , R_2 and R_3 is hydrogen or an alkyl group having 1 to 3 carbon atoms and X is oxygen or sulfur, or a pharmaceutically acceptable acid salt thereof which comprises condensing (I) a compound of the formula



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Rf value by thin layer chromatography on the same type of the plate with the same kind of the solvent as in Example 1 = 0.24.

Example 3.

Preparation of L-2-oxothiazolidine-4-carbonyl-L-histidyl-L-prolineamide

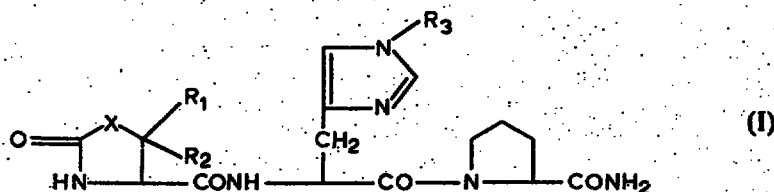
In 6 ml. of dimethylformamide, there are dissolved 260 mg. (2 mM) of L-2-oxothiazolidine-4-carboxylic acid and 650 mg. of L-histidyl-L-prolineamide dihydrochloride together with 271 mg. of N-hydroxybenztriazole. Under cooling with ice, 0.3 ml. of triethylamine and then 460 mg. of N,N'-dicyclohexylcarbodiimide are added. The mixture is stirred for 20 hours, after which time the reaction mixture is filtered. The filtrate is concentrated to dryness under reduced pressure and the concentrate is dissolved in 20 ml. of water. The insolubles, which are formed in minor amounts, are removed by filtration and the filtrate is adsorbed on a column (2x30cm) of carboxymethyl-Sephadex (Trade Mark). The column is then irrigated with aqueous solutions of ammonium acetate from water to 0.1 mole concentration (500 ml./500 ml.) by the gradient elution method. The fractions containing the contemplated product are pooled and lyophilized. The procedure gives 430 mg. of white powder.

Amino acid analysis: CysH:His:Pro = 0.73:1.00:0.98.

Rf value by thin layer chromatography on the same type of plate with the same kind of the solvent as in Example 1 = 0.20.

WHAT WE CLAIM IS:—

1. A compound of the formula.



wherein each of R_1 , R_2 and R_3 is hydrogen or an alkyl group having 1 to 3 carbon atoms and X is oxygen or sulfur, or a pharmaceutically acceptable acid salt thereof.

2. A compound as claimed in Claim 1, wherein R_1 is hydrogen and R_2 is an alkyl group having 1 to 3 carbon atoms.

3. A compound as claimed in Claim 1, wherein R_3 is an alkyl group having 1 to 3 carbon atoms.

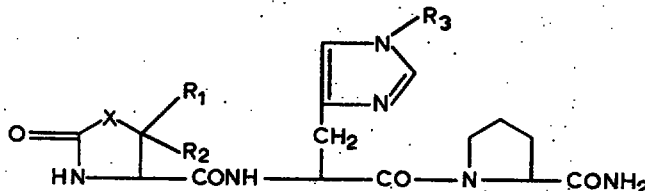
4. L-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

5. L-trans-5-methyl-2-oxo-oxazolidine-4-carbonyl-L-histidyl-L-prolineamide.

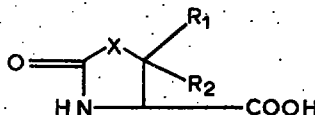
6. L-2-oxothiazolidine-4-carbonyl-L-histidyl-L-prolineamide.

7. A compound (I) as defined in claim 1 substantially as herein described with reference to any of the specific examples.

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



wherein each of R_1 , R_2 and R_3 is hydrogen or an alkyl group having 1 to 3 carbon atoms and X is oxygen or sulfur, or a pharmaceutically acceptable acid salt thereof which comprises condensing (1) a compound of the formula





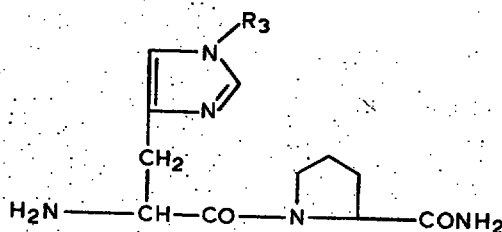
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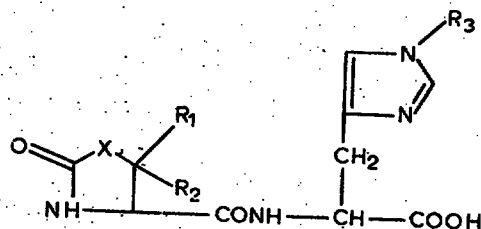
wherein R_1 , R_2 , and X have the same meaning as defined above with a compound of the formula



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wherein R_3 has the same meaning as defined above or (2) a compound of the formula

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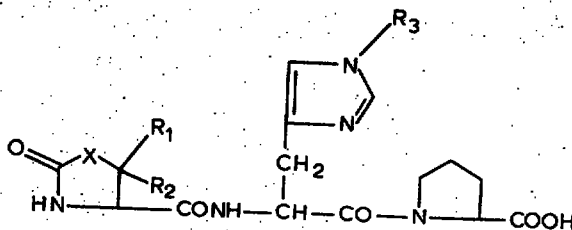
wherein R_1 , R_2 , R_3 , and X have the same meaning as defined above with a compound of the formula



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or (3) a compound of the formula

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wherein R_1 , R_2 , R_3 , and X have the same meaning as defined above with NH_2 , and, if desired reacting the resulting compound of general formula (I) with an acid to obtain a pharmaceutically acceptable salt of the compound (I).

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9. A process according to claim 8 substantially as herein described with reference to any of the specific examples.

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10. Compounds (I) when made by a process according to claim 8 or 9.

11. A pharmaceutical composition comprising a compound (I) as claimed in any of claims 1 to 7 and 10 together with a non-toxic pharmaceutically acceptable carrier or diluent therefor.

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12. A pharmaceutical composition according to claim 11 substantially as herein described.

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