

PATENT SPECIFICATION

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(54) NONAPEPTIDES

(71) We, AMERICAN HOME PRODUCTS CORPORATION, a corporation organised and existing under the laws of the State of Delaware, United States of America, of 685, Third Avenue, New York 10017, New York, United States of America, 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 nonapeptide analogues of LRH, to processes for their preparation, to pharmaceutical compositions containing them and to methods of treatment using them.

Many analogues of LRH have been recently produced and tested as agents for ovulation induction. Modification of the amino acid sequence of LRH has been most advantageous to date with removal of the Gly¹⁰ group and production of the Pro-NHC₂H₅ terminus, providing a compound reportedly three to five times as active as LRH itself. Fujino et al., Biochem. Biophys. Res. Commun. 49 863 (1972). D-Ala⁶-LRH was subsequently shown to be more potent than LRH, by Monahan et al., Biochemistry 12 4616 (1973). Various other D-amino acids have been inserted in 6-position of LRH and des-Gly¹⁰-Pro-NHC₂H₅-LRH to produce products with improved ovulation inducing properties. U.S. 3,913,412 and Vilchez-Martinez et al., Biochem. Biophys. Res. Commun. 59 1226 (1974). The potency of D-Ala⁶, des-Gly¹⁰-LRH ethylamide was reported by Coy et al., Biochem. Biophys. Res. Commun. 57 335 (1974) to be twice that of D-Ala⁶-LRH in stimulating LH secretion. Ling et al., Biochem. Biophys. Res. Commun. 63 801 (1975) report the synthesis and biological activity of D-Ala⁶, (N^a-Me)Leu⁷-LRH in stimulating the secretion of LH, which was found to possess 560% of LRH potency, placing it in Y is (N-methyl)Leu or (N-methyl)Ile

In accordance with this invention there is provided a group of nonapeptides of the formula:



in which

X is D-Trp or D-2-(1,4-cyclohexadienyl)Gly, and

Y is (N-methyl)Leu or (N-methyl)Ile

or a non-toxic acid addition salt or complex thereof. Of these compounds, [D-Trp⁶, L-(N-methyl)Leu⁷, Des-Gly-NH₂¹⁰, Pro-ethylamide]LRH is the preferred species from its activity standpoint. These compounds induce ovulation in animals and are claudogenic-interceptive agents useful in preventing or terminating pregnancy in mammals. The compounds act as claudogenic-interceptive agents when administered post-coitally to a female mammal after ovulation, in that they disrupt the normal physiological processes necessary for implantation and/or maintenance of a fertilized ovum.

The intermediates employed in the production of the nonapeptides of this invention form an additional aspect of the invention. The intermediates are the fully or partially protected polypeptide-resin and the fully or partially protected polypeptide ethylamides of the formula:

H-Pyrroglu-His(R)-Trp-Ser(R¹)-Tyr(R²)-X-Y-Arg(R³)-Pro-Z (II)

in which

Z represents OH or an acylating derivative thereof, including an ester, e.g. lower alkyl ester, benzyl ester or ester of formula —OCH₂[polystyrene resin support] or —NHC₂H₅; X and Y are as defined above, preferably X is D-Trp and Y is L-(N-methyl)Leu; R is hydrogen or a protecting group for the imino nitrogen of the histidyl moiety and R³ is hydrogen or one or more protecting groups for the guanyl function of the arginyl moiety; for example R and R³ may be tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl or nitro. The tosyl group is preferred as the protecting group for both R and R³. However, the guanyl group of the arginyl moiety may be protected via the N^ω or N^{ω1} nitrogen atoms by the nitro or tosyl protecting groups and via the N^δ nitrogen atom or either of the N^ω or N^{ω1} nitrogen atoms by the benzyloxycarbonyl, adamantyloxycarbonyl or trityl group; and R¹ and R² each are hydrogen or a protecting group for the hydroxyl groups of serine and tyrosine. The hydroxyl protecting groups employed for this purpose may be acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl and 2,6-dichlorobenzyl, the benzyl and 2,6-dichlorobenzyl groups being preferred for this purpose; with the proviso that at least one of R, R¹, R² and R³ is other than hydrogen.

Preferably R and R³ are tosyl, Preferably R¹ is benzyl and R² is 2,6-dichlorobenzyl.

The term "lower" as used herein in connection with a group or molecule means the group or molecule contains 1 to 6 carbon atoms.

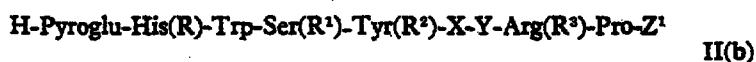
By the term "acylating derivative" as used herein is meant a derivative of the peptide acid having a C-terminal function which can be used to acylate an amine to form an amide.

This invention further provides processes for preparing nonapeptides of formulae I and II. One such process for preparing the nonapeptides of formula I as defined above comprises deprotecting a corresponding nonapeptide of formula:



wherein R, R¹, R², R³, X and Y are as defined above and if desired converting the product to the free base or a non-toxic acid addition salt or a complex. The deprotection may be carried out by techniques generally known in the art, preferably in a single step using liquid hydrogen fluoride in the presence of anisole.

The protected peptide of formula II(a) may be prepared by acylating ethylamine with a suitably protected nonapeptide of formula:



wherein R, R¹, R², R³, X and Y are as defined above and Z¹ represents OH or an acylating derivative thereof and if desired removing one or more of the protecting groups. Examples of acylating derivatives include esters, e.g. lower alkyl or benzyl esters or an ester formed with a resin support in which case Z¹ represents —OCH₂[polystyrene resin support]. The acylation may be carried out by known methods, for example when Z¹ is OH, by using a condensing agent such as dicyclohexylcarbodiimide.

The compounds of formula II(b) wherein Z¹ is OH or an acylating functional derivative thereof may be prepared by known methods for building up an amino acid sequence as described in standard textbooks on peptide synthesis. When a nonapeptide wherein Z¹ is —OCH₂[polystyrene resin support] is required then this compound may be prepared by solid phase methodology, following techniques generally known in the art for building an amino acid sequence from an initial resin supported amino acid. Merrifield, J.A.C.S. 85, 2149 (1963) generally illustrates the technique involved.

The resin support employed may be any suitable resin conventionally employed in the art for the solid phase preparation of polypeptides, preferably a copolymer of styrene and divinyl benzene in which the degree of crosslinking by the divinyl benzene is from 0.5 to 3%; which resin has been chloromethylated to provide sites for ester formation with the initially introduced protected amino acid. The amino protected proline may be coupled to the chloromethylated resin according to the pro-

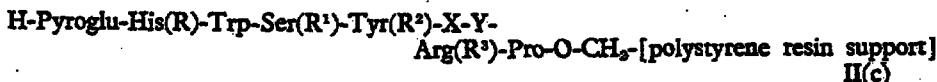
cedure of Gisin, *Helv. Chim. Acta.*, 56, 1476 (1973). Following the coupling of the amino protected proline to the resin support, the amino protecting group may be removed by standard methods employing trifluoroacetic acid in methylene chloride, trifluoroacetic acid alone or HCl in dioxane. The deprotection may be carried out at a temperature between 0°C and room temperature. After removal of the amino protecting group the remaining α -amino protected and, if necessary, side chain protected amino acids are coupled, *seriatim*, in the desired order to obtain the product. Alternatively, multiple amino acid group may be coupled by the solution method prior to coupling with the resin supported amino acid sequence. The selection of an appropriate coupling reagent is within the skill of the art. A particularly suitable coupling reagent is N,N'-diisopropylcarbodiimide. Another applicable coupling agent is N,N'-dicyclohexylcarbodiimide.

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in a two to six fold excess and the coupling is carried out in a medium of dimethylformamide: methylene chloride or in either dimethylformamide or methylene chloride alone. In cases where incomplete coupling occurs the coupling procedure is repeated before removal of the α -amino protecting group, prior to introduction of the next amino acid to the solid phase reactor. The success of the coupling reaction at each stage of the synthesis is monitored by the ninhydrin reaction as described by E. Kaiser et al., *Analyt. Biochem.*, 34, 595 (1970).

The necessary α -amino protecting group employed for each amino acid introduced into the polypeptide is preferably *tert*-butoxycarbonyl, although any such protecting group may be employed as long as it is not removed under coupling conditions and is readily removed selectively in relation to the other protecting groups present in the molecule under conditions which otherwise do not affect the formed molecule. Additional examples of such α -amino protecting groups from which selection may be made, after consideration of the rest of the polypeptide molecule, are trityl, phthalyl, tosyl, allyloxycarbonyl, cyclopentylloxycarbonyl, *tert*-amyloxycarbonyl, benzyloxycarbonyl and *o* or *p*-nitrobenzyloxycarbonyl.

The criterion for selecting protecting groups for R—R² are (a) the protecting group must be stable to the reagent and under the reaction conditions selected for removing the α -amino protecting group at each step of the synthesis, (b) the protecting group must retain its protecting properties (i.e. not be split off under coupling conditions), and (c) the protecting group must be readily removable upon conclusion of the polypeptide synthesis, under conditions that do not otherwise affect the polypeptide structure.

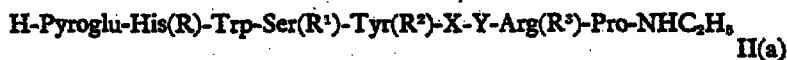
The fully protected, resin supported nonapeptides present the amino acid sequence:



in which the group -O-CH₂-[polystyrene resin support] represents the ester moiety of one of the many functional groups present in the polystyrene resin.

Accordingly this invention also provides a process for preparing a compound of formula II(c) as defined above which comprises reacting the requisite amino acids, protected and/or activated as necessary, sequentially with a chloromethylated polystyrene resin support using solid phase methodology.

The fully protected nonapeptides are preferably removed from their resin support by treatment with ethylamine at room temperature followed by removal of any excess ethylamine to yield the intermediate.



Preferably the fully protected intermediate described in the preceding paragraph is deprotected with liquid hydrogen fluoride in the presence of anisole to yield the nonapeptide claudogenic-interceptive agents of this invention.

Alternatively the resin supported nonapeptides of formula II(c) may be cleaved by transesterification to give an ester, e.g. a lower alkyl or benzyl ester by transesterification with a lower alkanol or benzyl alcohol, in the presence of a base such as triethylamine. These ester derivatives of formula II(b) may be used to prepare the intermediate of formula II(a) as hereinbefore described.

A further method for preparing the protected nonapeptide of formula II(a)

comprises coupling the requisite amino acids, protected and/or activated as necessary, and ethylamine in any order of succession to give the desired peptide sequence, and if desired removing one or more of the protecting groups.

The coupling of the amino acids and ethylamine in the above mentioned process may be carried out by the standard methods used in peptide chemistry. Such methods are described in the literature for example in standard textbooks on peptide synthesis—see for example Schroder and Lubke, "The Peptides", Academic Press 1965 and Greenstein and Winitz, "Chemistry of the Amino Acids" Vol. 2, John Wiley and Sons Inc., 1961. An example of a process for preparing a compound of formula II(a) starting from constituent amino acids and ethylamine is shown in the scheme below. Standard abbreviations for amino acids, protecting groups and coupling agents have been employed in the scheme—for example see Schroder and Lubke, *supra*, pp xiii—xxix. The term "azide" used in the scheme refers to the azide method for coupling amino acids, thus the first step involves the formation of dipeptide H-pyroglu-His-OMe by condensation using DCC (dicyclohexylcarbodiimide) of H-Pyroglu-OH and His-OMe. The dipeptide is reacted with hydrazine to give the tripeptide H-Pyroglu-His-Trp-OBzl. By coupling further peptide fragments the NO₂-protected peptide of formula II(a) is formed, which is deprotected as shown in the final step, to give a compound of formula I.

The acid addition salts of the nonapeptides of this invention are produced by known techniques from either inorganic or organic acids known to afford pharmaceutically acceptable non-toxic addition products, such as hydrochloric, hydrobromic, sulfuric, phosphoric, maleic, acetic, citric, benzoic, succinic, malic and ascorbic acid.

The claudogenic-interceptive agents of this invention, when administered to a female mammal, postcoitally pursuant to a daily regimen of at least 3 micrograms per day per kilogram host body weight for a period of at least three days, completely prevent reproduction.

Although applicants do not wish to be bound by any specific theory of activity, they propose and believe, based upon studies conducted with a variety of animal models, that the nonapeptides of this invention exert a claudogenic/interceptive action via stimulation of the hypophysial-ovarian steroid axis.

In any event, regardless of the physiological pathway to the end result, the nonapeptides of this invention effectively prevent reproduction in female mammals upon administration after coitus.

Hence the nonapeptides of this invention are useful as "morning-after" contraceptives to prevent reproduction in the female mammal. Within this context, the nonapeptides may be used as anti-littering agents for control of rodent populations without use of rodenticides and their possible undesirable effect on other animals in the environment.

Reproduction was avoided in the animal models by daily administration of a nonapeptide of this invention during the day 1 to day 7 period after coitus as well as upon daily administration over the 7 to day 12 period, post coitus. Thus both a claudogenic (pre-implantation) as well as an interceptive (post-implantation) type of interference with pregnancy was established.

The procedure followed in evaluating the anti-gravidity properties of the nonapeptides of this invention was as follows:

Mature, female, Sprague-Dawley rats (350 ± 30 grams body weight) maintained on a 14:10 light:dark schedule were caged with fertile male rats on the evening of proestrus. The presence of vaginal sperm the next morning was considered day 1 of pregnancy. The nonapeptide ethylamide produced in the following examples, representative of the entire group of compounds of this invention, was administered subcutaneously in a corn oil vehicle on days 1-7, or 7-12 of pregnancy at a rate of as low as 1 $\mu\text{g}/\text{rat}/\text{day}$. One-half the daily dose was administered at 9 a.m. and at 3 p.m. each day. The recipients of day 1-7 treatment were autopsied on day 14. The recipients of day 7-12 treatment were autopsied on day 18 of pregnancy. The effectiveness of the ethylamide and its effective dose was established by the absence of uterine implantation sites and fetuses. The presence of at least one normal fetus was considered to be the criterion for pregnancy. The claudogenic interceptive activity of the nonapeptides of this invention was thereby established at a daily dose as low as about 3 micrograms per kilogram host body weight, the treatment being 100 per cent effective at a dose of 1 microgram per rat per day in a five rat sample for the day 1-7 period and 10 micrograms for the day 7-12 period.

For the purpose of defining the post coital stages of pregnancy in the rat as an experimental model, the following schedule is provided in definition of post-coital contraceptive activity which, for the purpose of this disclosure, is intended to embrace both pre-claudogenic and post-(interceptive) implantation contraceptive activity; day 1-vaginal sperm; days 1-3-ova transport in oviducts, fertilization; days 3-5-blastocyst free in uterine lumen; days 5-7-implantation into uterine wall; days >7-post implantation.

Based upon the findings of activity in the prevention of development of pregnancy in the rat model and the fact that present evidence indicates that the hormonal situation relating to the reproductive cycle up to and including ovulation, is basically the same in all female vertebrates, e.g. the human reproductive cycle is physiologically analogous with that of the rat, the activity of the nonapeptides of this invention effectively interferes with the development of the blastocyst pre- and post-implantation in the uterus in all mammals, including the human.

Thus, in accordance with the use aspect of this invention there is provided a method for preventing reproduction in a mammal-excluding humans which comprises administering a compound of formula I to said mammal for a time and in an amount sufficient to prevent reproduction. Typically the compound of formula I is administered post coitally, in a daily regimen containing at least 3 micrograms per kilogram host body weight for a time sufficient to prevent reproduction. In operation, the anti-gravidity compounds of this invention interfere with the mechanism of gestation, whether that interference is by an early post-coital, pre-implantation

contraceptive or by post-implantation interceptive mechanism. This invention also provides a method for preventing the implantation of a fertilized ovum in a female mammal which comprises administering a compound of formula I for a time and in an amount sufficient to prevent implantation. The effective sequence of daily administration in the human is from day 1 of ovulation-fertilization to day 6 to produce a claudogenic response. The human dose, based upon the posology of the experimental model, is approximately 1.5 milligrams per day for a fifty kilogram female. For practical purposes, the applicable subcutaneous human dose is about 50 μ g/kg/day or 2.5 mg/50 kg female for a claudogenic effect.

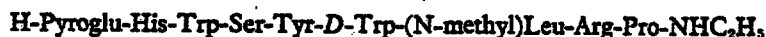
This invention also provides a pharmaceutical composition comprising a compound of formula I, or a non-toxic acid addition salt or complex thereof, in association with a pharmaceutically acceptable carrier. Conventional complexes of the nonapeptides may be employed to prolong their effectiveness, such as the protamine zinc or aluminium complexes which are prepared by conventional techniques. Preferably the pharmaceutical composition is in unit dosage form. This invention also provides a method for preparing a pharmaceutical composition which comprises bringing the compound of formula I or non-toxic acid addition salt thereof into association with a pharmaceutically acceptable carrier.

A preferred method for preventing reproduction comprises administering said nonapeptide orally or parenterally, to said mammal following a daily regimen beginning on day one after coitus and extending through about the first eight days post implantation, or through about day seven post coitus.

The ovulation-induction properties of the nonapeptides of this invention was determined with the preferred compound, [D-Trp⁶, (N-methyl)Leu⁷, des-Gly-NH₂¹⁰, Pro-ethylamide]LRH as follows:

Proestrous female Sprague-Dawley rats were injected intraperitoneally with a hypnotic dose of Nembutal (50 mg/kg) at 1:30 P.M. (Nembutal is a Registered Trade Mark). Between 1:40 and 1:50 p.m. the rats received the test material via the jugular vein. The following morning the animals were sacrificed and the fallopian tubes examined for ova under a dissecting microscope. The results of this test demonstrated ovulation inducing activity in 100 percent of the rats at a dose of 0.1 mg per rat, in comparison with LRH which requires about 3 mg per rat to obtain 100 percent ovulation and [D-Ala⁶, des-Gly-NH₂¹⁰, Pro-ethylamide]LRH which requires about 1 mg per rat for the same response. Thus, in its ovulation induction, the preferred compound of this invention is about ten times as active as its closest structural analogues.

The following examples illustrate the preparation of



which is the preferred claudogenic-interceptive agent of this invention. It is to be understood, that the other nonapeptides disclosed herein are prepared in the same manner by merely substituting the indicated specific amino acid into the preparatory sequence at the proper position. Thus, the examples are illustrative rather than limiting in nature.

Example 1

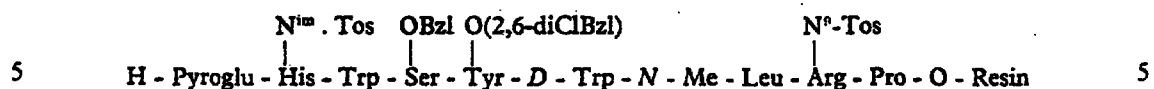
Preparation of tert-butyloxycarbonyl-L-proline resin (method of Gisin, *Helv. Chim. Acta*, 56, 1476 [1973])

Tert-Butyloxycarbonyl-L-proline (18.56 g., 86.3 m moles) in an ethanol (112 ml.)—water (48 ml.) mixture was treated with concentrated aqueous cesium hydrogen carbonate solution until the pH of the solution reached 7. The reaction mixture was stripped and dried by repeated stripping using ethanol, ethanol-benzene, benzene (three times). The residue was dried over phosphorous pentoxide, *in vacuo* at room temperature overnight.

The total product in dimethylformamide (880 ml.) was stirred overnight at 50°C, under nitrogen, with 80 g. of Bio-Beads S. X. 1 Resin (chloromethylated capacity 0.89 meq./g.). The filtered resin was washed thoroughly with dimethylformamide (twice), dimethylformamide—10% v/v water (twice), dimethylformamide (twice), methanol (twice), chloroform (thrice) and dried over P₂O₅. Amino acid analysis indicated a substitution on the resin of 0.56 meq./g.

Example 2

L-Pyroglutamyl-N^{im}-tosyl-L-histidyl-L-tryptophyl-O-benzyl-L-seryl-O-(2,6-dichlorobenzyl)-L-tyrosyl-D-tryptophyl-N-methyl-L-leucyl-N^o-tosyl-L-arginyl-L-proline resin ester



The tert-butyloxycarbonyl-L-proline resin (4.725 g) from Example 1 was placed in a Beckman 990 peptide synthesizer (Beckman is a Registered Trade Mark) and treated as follows:

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| 10 | <ol style="list-style-type: none"> 1. Wash with methanol. 2. Wash with methylenechloride. 3. Wash with methanol (two times). 4. Wash with methylenechloride (two times). 5. Prewash with 1:1 trifluoroacetic acid-methylchloride (v/v containing 0.5% (w/v) dithioerythritol). | 10 |
| 15 | <ol style="list-style-type: none"> 6. Deprotection with the solvent from step 5 (two times for 15 minutes each). 7. Wash with methylenechloride. 8. Wash with dimethylformamide. 9. Wash with 12.5% triethylamine in dimethylformamide (v/v) for 10 minutes (two times). | 15 |
| 20 | <ol style="list-style-type: none"> 10. Wash with dimethylformamide. 11. Wash with methylene chloride (two times). 12. Wash with methanol (two times). 13. Wash with methylene chloride (three times). | 20 |
| 25 | <ol style="list-style-type: none"> 14. The peptide resin is then gently stirred with 9 moles of the desired tert-butyloxycarbonyl amino acid in ca. 30 ml. of a 1:1 dimethylformamide-methylene chloride (v/v) solvents. 15. 1M diisopropylcarbodiimide (DIC) in methylene chloride (10 m moles) is added in two portions over 30 minutes. 16. The reaction mixture is stirred for 7 hours. | 25 |

30	A contact time of 1.5 minutes is allowed for each step unless otherwise stated. The above stated steps are repeated until all of the desired amino acids are added.	30
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35	The following amino acid residues were then introduced consecutively: t-Boc-N^o-tosyl-L-arginine (9 m moles), t-Boc-N-methyl-L-leucine (9 m moles), t-Boc-D-tryptophan (9 m moles), then without deblocking a second portion of t-Boc-D-tryptophan (9 m moles) was allowed to react. Then resuming the normal sequence t-Boc-2,6-dichlorobenzyl-L-tyrosine (9 m moles), t-Boc-O-benzyl-L-serine (9 m moles), t-Boc-L-tryptophan (9 m moles), t-Boc-im-tosyl-L-histidine (9 m moles), and L-2-pyrrolidone-5-carboxylic acid (9 m moles) were added. The washed peptide resin was dried <i>in vacuo</i> and weighed 8.422 g.	35
40		40

Example 3

L-pyroglutamyl-N^{im}-tosyl-L-histidyl-L-tryptophyl-O-benzyl-L-seryl-O-(2,6-dichlorobenzyl)-L-tyrosyl-D-tryptophyl-N-methyl-L-leucyl-N^o-tosyl-L-arginyl-N-ethyl-L-prolinamide

45	Protected peptide-resin (8.44 g) from Example 2 and ethylamine (120 ml.) were stirred overnight in a glass pressure bottle. Ethylamine was removed under reduced pressure and the residue washed with methanol dimethylformamide (four times), methanol and methylene chloride. The combined filtrates were evaporated <i>in vacuo</i> below 35°C to give the title compound (3.277 g).	45
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Example 4

L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-tryptophyl-N-methyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide

50	The product of Example 3 was treated <i>in vacuo</i> with anhydrous liquid hydrogen fluoride (120 ml.) and anisole (35 ml.) for 50 minutes at 0°C. Hydrogen fluoride was removed under reduced pressure and the residue distributed between diethyl ether and 10% (v/v) aqueous acetic acid. Lyophilization of the acid layer afforded the crude title product (2.404 g.).	50
55		55

Example 5

Purification and characterisation of L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-tryptophyl-N-methyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide

The crude peptide (2.404 g.) in a minimum volume of 0.2N acetic acid was applied to a column of Bio Gel P-2 previously equilibrated with 0.2N acetic acid and then eluted with the same solvent. Fractions of 9 ml. each were collected. Peptide material was located by Ehrlich spot test and UV analysis. One major fraction was obtained 34—50 (2.234 g.). A second Bio-Gel P-2 column afforded 1.928 g. of desired material. This material was rechromatographed on a partition column of Sephadex G-25 fine (2.5×100 cm), prepared by equilibration with lower phase and then upper phase of the BAW system (n-butanol:acetic acid:water, 4:1:5 by volume). (Sephadex is a Registered Trade Mark). Elution with upper phase afforded fractions A 36—48 (820 mg.) B 49—64 (633 mg.). Fraction A was rechromatographed using the same system which afforded 295 mg. of the desired peptide.

The R_f value of the peptide (30 µg load) in the thin layer system (silica, plates-Brinkman) n-butanol:acetic acid:water (4:1:5 by volume, upper phase). R_f 0.21 n-butanol:acetic acid; ethyl acetate:water (1:1:1 by volume). R_f 0.57.

The optical rotation measured on a Carl Zeiss LEP A-2 photoelectric precision polarimeter, $[\alpha]_D^{25} = -80.91^\circ$ (c=0.98, 1% (v/v) acetic acid). (Carl Zeiss is a Registered Trade Mark).

Hydrolysis of the peptide in methanesulfonic acid (0.2 ml./1 mg. peptide) for 20 hours at 110°C. in a closed evacuated system revealed all of the required amino acids to be present.

We are aware of the Abortion Act 1967.

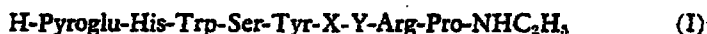
We make no claim to methods of preventing reproduction which are contrary to law.

We make no claim to methods for the treatment or prevention of disease in humans.

Subject to the foregoing disclaimers

WHAT WE CLAIM IS:—

1. A compound having the formula



in which X is D-Trp or D-2-(1,4-cyclohexadienyl)Gly and Y is (N-methyl)Leu or (N-methyl)Ile, or a non toxic acid addition salt or complex thereof.

2. A compound having the formula



in which Z represents OH or an acylating derivative thereof as hereinbefore defined, or $\text{—NHC}_2\text{H}_5$; X and Y are as defined in Claim 1; R is hydrogen or a protecting group for the imino nitrogen of the histidyl moiety; R' is hydrogen or one or more protecting groups for the guanyl function of the arginyl moiety; and R' and R'' each are hydrogen or a protecting group for the hydroxyl groups of serine and tyrosine, with the proviso that at least one of R, R', R'' and R''' is other than hydrogen.

3. A compound as claimed in claim 2 in which the acylating derivative is an ester wherein Z is —O— (lower alkyl) as hereinbefore defined or —O—benzyl .

4. A compound of formula II as claimed in claim 2 or claim 3 wherein R is tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl or nitro.

5. A compound of formula II as claimed in any one of claims 2 to 4 wherein R' is tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl or nitro.

6. A compound of formula II as claimed in any one of claims 2 to 5 wherein R' and R'' represents acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl or 2,6-dichlorobenzyl.

7. A compound of formula II as claimed in claim 2 wherein Z is $\text{—NHC}_2\text{H}_5$, R and R' are independently selected from acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl and nitro; and R' and R'' are independently selected from acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl and 2,6-dichlorobenzyl.

8. A compound as claimed in claim 2 in which the acylating derivative is an

Example 5

Purification and characterisation of L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-tryptophyl-N-methyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide

The crude peptide (2.404 g.) in a minimum volume of 0.2N acetic acid was applied to a column of Bio Gel P-2 previously equilibrated with 0.2N acetic acid and then eluted with the same solvent. Fractions of 9 ml. each were collected. Peptide material was located by Ehrlich spot test and UV analysis. One major fraction was obtained 34—50 (2.234 g.). A second Bio-Gel P-2 column afforded 1.928 g. of desired material. This material was rechromatographed on a partition column of Sephadex G-25 fine (2.5×100 cm), prepared by equilibration with lower phase and then upper phase of the BAW system (n-butanol:acetic acid:water, 4:1:5 by volume). (Sephadex is a Registered Trade Mark). Elution with upper phase afforded fractions A 36—48 (820 mg.) B 49—64 (633 mg.). Fraction A was rechromatographed using the same system which afforded 295 mg. of the desired peptide.

The Rf value of the peptide (30 µg load) in the thin layer system (silica, plates-Brinkman) n-butanol:acetic acid:water (4:1:5 by volume, upper phase). Rf 0.21 n-butanol:acetic acid; ethyl acetate:water (1:1:1:1 by volume). Rf 0.57.

The optical rotation measured on a Carl Zeiss LEP A-2 photoelectric precision polarimeter, $[\alpha]_D^{25} = -80.91^\circ$ (c=0.98, 1% (v/v) acetic acid). (Carl Zeiss is a Registered Trade Mark).

Hydrolysis of the peptide in methanesulfonic acid (0.2 ml./1 mg. peptide) for 20 hours at 110°C. in a closed evacuated system revealed all of the required amino acids to be present.

We are aware of the Abortion Act 1967.

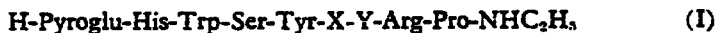
We make no claim to methods of preventing reproduction which are contrary to law.

We make no claim to methods for the treatment or prevention of disease in humans.

Subject to the foregoing disclaimers

WHAT WE CLAIM IS:—

1. A compound having the formula



in which X is D-Trp or D-2-(1,4-cyclohexadienyl)Gly and Y is (N-methyl)Leu or (N-methyl)Ile, or a non toxic acid addition salt or complex thereof.

2. A compound having the formula



in which Z represents OH or an acylating derivative thereof as hereinbefore defined, or $\text{—NHC}_2\text{H}_5$; X and Y are as defined in Claim 1; R is hydrogen or a protecting group for the imino nitrogen of the histidyl moiety; R^1 is hydrogen or one or more protecting groups for the guanyl function of the arginyl moiety; and R^1 and R^2 each are hydrogen or a protecting group for the hydroxyl groups of serine and tyrosine, with the proviso that at least one of R, R^1 , R^2 and R^3 is other than hydrogen.

3. A compound as claimed in claim 2 in which the acylating derivative is an ester wherein Z is —O— (lower alkyl) as hereinbefore defined or —O—benzyl .

4. A compound of formula II as claimed in claim 2 or claim 3 wherein R is tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl or nitro.

5. A compound of formula II as claimed in any one of claims 2 to 4 wherein R^3 is tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl or nitro.

6. A compound of formula II as claimed in any one of claims 2 to 5 wherein R^1 and R^2 represents acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl or 2,6-dichlorobenzyl.

7. A compound of formula II as claimed in claim 2 wherein Z is $\text{—NHC}_2\text{H}_5$, R and R^3 are independently selected from acetyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl, adamantyloxycarbonyl and nitro; and R^1 and R^2 are independently selected from acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl and 2,6-dichlorobenzyl.

8. A compound as claimed in claim 2 in which the acylating derivative is an



* C L N S *

ester wherein Z is OCH₂[polystyrene resin support], R and R³ are independently selected from tosyl, acetyl, benzoyl, *tert*-butyl, trityl, benzyl benzyloxycarbonyl, adamantyloxycarbonyl and nitro, R¹ and R² are independently selected from acetyl, tosyl, benzoyl, *tert*-butyl, trityl, benzyl, benzyloxycarbonyl and 2,6-dichlorobenzyl.

5 9. A compound as claimed in claim 8 wherein the polystyrene resin is a copolymer of styrene and divinyl benzene in which the degree of cross-linking by the divinyl benzene is 0.5 to 3%.

10 10. A compound as claimed in any one of claims 6 to 8 wherein R¹ and R³ are tosyl, R is benzyl and R² is 2,6-dichlorobenzyl.

11. A compound as claimed in any one of claims 1 and 6 to 9 wherein X is *D*-Trp.

12. A compound as claimed in any one of claims 1 and 6 to 9 wherein Y is *L*-(*N*-methyl)Leu.

15 13. *L*-pyroglutamyl-*L*-histidyl-*L*-tryptophyl-*L*-seryl-*L*-tyrosyl-*D*-tryptophyl-*L*-(*N*-methyl)leucyl-*L*-arginyl-*N*-ethyl-*L*-prolinamide or a non-toxic acid addition salt thereof.

14. A compound as claimed in claim 1 or claim 13 in which the acid addition salt is the hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate or ascorbate salt.

20 15. A process for preparing a compound of formula I as defined in claim 1 which comprises deprotecting a corresponding compound of formula



wherein X and Y are as defined in claim 1 and R, R¹, R², and R³ are as defined in claim 2, and if desired converting the product to the free base or to a non-toxic acid addition salt or complex thereof.

25 16. A process as claimed in Claim 15 which is effected using liquid hydrogen fluoride in the presence of anisole.

17. A process as claimed in claim 15 or claim 16 in which R, R¹, R² and R³ are as defined in any of claims 4 to 6.

30 18. A process as claimed in claim 15 in which R, R¹, R² and R³ are as defined in claim 17.

19. A process as claimed in claim 18 wherein X is *D*-Trp.

20. A process as claimed in claim 18 or 19 where Y is *L*-(*N*-methyl)Leu.

21. A process for preparing a compound of formula

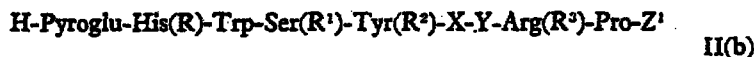


35 wherein R, R¹, R² and R³ are as defined in claim 2 and X and Y are as defined in claim 1, which comprises reacting the requisite amino acids, protected and/or activated as necessary, and ethylamine in any order of succession to give the desired peptide sequence, and if desired removing one or more of the protecting groups.

22. A process for preparing a compound of formula



wherein X and Y are as defined in claim 1 and R, R¹, R² and R³ are as defined in claim 2, which comprises acylating ethylamine with a protected compound of formula:



45 wherein R, R¹, R², R³, X and Y are as defined above and Z¹ represents OH or an acylating derivative thereof, and if desired removing one or more of the protecting groups.

23. A process as claimed in claim 22 in which the acylating derivative is an ester wherein Z¹ represents —OCH₂[polystyrene resin support].

50 24. A process for preparing a compound of formula II(b) as defined in claim 22 wherein Z¹ is —OCH₂[polystyrene resin support] which comprises reacting the requisite amino acids, protected and/or activated as necessary, sequentially with a chloromethylated polystyrene resin support using solid phase methodology.



* C L M S *

25. A process as claimed in claim 23 or claim 24 wherein R, R¹, R² and R³ are as defined in claim 7.

26. A process as claimed in any one of claims 15 to 17 wherein the compound of formula II(a) is prepared by a process as claimed in any one of claims 21 to 23.

27. A process as claimed in claim 23 in which the compound of formula II(b) is prepared by a process as claimed in claim 24 or 25.

28. A process for preparing a compound of formula I as defined in claim 1 substantially as hereinbefore described with reference to Example 4.

29. A process for preparing a compound of formula II(a) as defined in claim 21 substantially as hereinbefore described with reference to Example 3.

30. A process for preparing a compound of formula II(b) as defined in claim 22 substantially as hereinbefore described with reference to Example 1 and 2.

31. A compound of formula I as defined in claim 1 whenever prepared by a process as claimed in any one of claims 15 to 17 and 26.

32. A compound of formula I as defined in claim 1 whenever prepared by a process as claimed in any one of claims 18 to 20, 27 and 28.

33. A compound of formula II as defined in claim 2 whenever prepared by a process as claimed in any one of claims 21 to 23.

34. A compound of formula II as defined in claim 2 whenever prepared by a process as claimed in any one of claims 24, 25, 29 and 30.

35. A pharmaceutical composition comprising a compound of formula I, or a non-toxic acid addition salt or complex thereof, as claimed in any one of claims 1 and 11 to 14, in association with a pharmaceutically acceptable carrier.

36. A pharmaceutical composition as claimed in claim 35 wherein the compound of formula I is in the form of the protamine zinc or aluminium complex.

37. A pharmaceutical composition as claimed in claim 35 or claim 36 when in unit dosage form.

38. A method for preparing a pharmaceutical composition as claimed in claim 35 or claim 36 which comprises bringing the compound of formula I or non-toxic acid addition salt or complex thereof into association with a pharmaceutically acceptable carrier.

39. A method for preventing the implantation of a fertilized ovum in a female mammal which comprises administering a compound as claimed in claim 1 for a time and in an amount sufficient to prevent implantation.

40. A method for preventing reproduction in a mammal excluding humans, which comprises administering a compound as claimed in claim 1 for a time and in an amount sufficient to prevent reproduction.

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