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(54) Title: METHOD FOR PRODUCING PEPTIDE SALTS, THEIR USE, AND PHARMACEUTICAL PREPARATIONS CONTAINING THESE PEPTIDE SALTS

(54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG VON PEPTIDSALZEN, DEREN VERWENDUNG UND DIE PEPTIDSALZE ENTHALTENDE PHARMAZEUTISCHE ZUBEREITUNGEN

(57) Abstract: The invention relates to pharmaceutical preparations containing peptide salt, to their production, and to the use thereof. The invention particularly relates to pharmaceutical preparations containing a slightly soluble salt of LHRH agonists or antagonists such as cetrorelix embonate for the parenteral administration in mammals with a long-sustained action.

(57) Zusammenfassung: Die Erfindung betrifft Peptidsalz enthaltende pharmazeutische Zubereitungen, deren Herstellung und deren Verwendung. Insbesondere betrifft die Erfindung pharmazeutische Zubereitungen enthaltend ein schwerlösliches Salz von LHRH Agonisten oder Antagonisten, wie Cetrorelix Embonat, zur parenteralen Applikation bei Säugetieren mit langanhaltender Wirkung.

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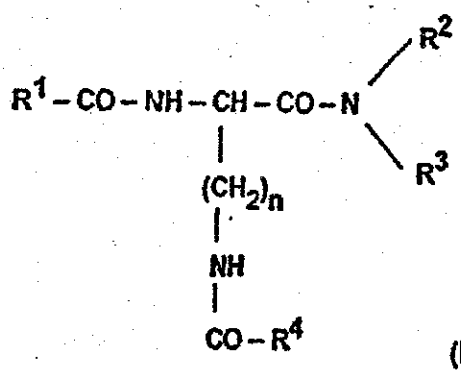
Method For Producing Peptide Salts, Their Use, And Pharmaceutical Preparations Containing These Peptide Salts

Description

The present invention relates to a process for the production of a pharmaceutical preparation for parenteral administration to mammals, characterized in that an acid addition salt of a basic peptidic LHRH antagonist (starting peptide salt) (1) is reacted in the presence of a suitable diluent with a mixed bed ion exchanger or a mixture of an acidic and basic ion exchanger with formation of the free basic peptide, the ion exchanger is subsequently separated off and the free basic peptide is then reacted with an inorganic or organic acid with formation of the desired acid addition salt of the peptide (final peptide salt) (2), suitable pharmaceutical excipients, vehicles and/or bulking agents are then added and the diluent is subsequently removed.

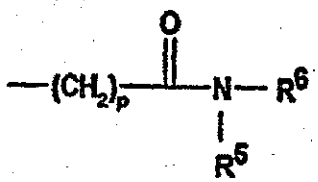
In the international patent application No. WO 95/15767, the preparation of a poorly soluble peptide by reaction of an aqueous solution of the acid salt with an acetic acid solution of the basic peptide with precipitation of the poorly soluble acid addition salt of the peptide is described. For example, the preparation of the LHRH antagonist cetrorelix embonate is described.

The expression "basic peptidic LHRH antagonists" here means antide, A-75998, ganirelix, NaI-Glu antagonist, cetrorelix, teverelix (Antarelix®) and the antagonists as per the compounds: the US 5,942,493 compounds of the general formula I



(I)

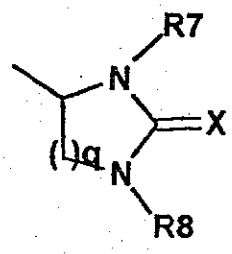
in which n is the number 3 or 4, R^1 is an alkyl group, an alkyloxy group, an aryl group, a heteroaryl group, an aralkyl group, a heteroaralkyl group, an aralkyloxy group or a heteroaralkyloxy group, in each case unsubstituted or substituted, R^2 and R^3 independently of one another are each a hydrogen atom, an alkyl group, an aralkyl group or a heteroaralkyl group, in each case unsubstituted or substituted, where the substitution can in turn consist of an aryl group or heteroaryl group, or $-NR^2R^3$ is an amino acid group, and R^4 is a group having the formula (II)



(II)

in which p is an integer from 1 to 4, R^5 is hydrogen or an alkyl group and R^6 is an unsubstituted or substituted aryl group or heteroaryl group,

or R^4 is a ring of the general formula (III)



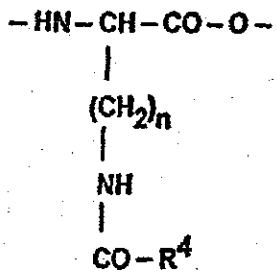
(III)

in which q is the number 1 or 2, R^7 is a hydrogen atom or an alkyl group, R^8 is a hydrogen atom or an alkyl group and X is an oxygen or sulphur atom, where the aromatic or heteroaromatic radicals can be partially or completely hydrogenated and chiral carbon atoms can have the R- or S-configuration, and their salts with pharmaceutically acceptable acids,

the compound of the general formula V

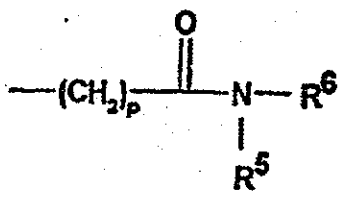
$$Ac\text{-}D\text{-}Nal(2)^1\text{-}D\text{-}(pCl)Phe^2\text{-}D\text{-}Pal(3)^3\text{-}Ser^4\text{-}Tyr^5\text{-}D\text{-}Xxx^6\text{-}Leu^7\text{-}Arg^8\text{-}Pro^9\text{-}D\text{-}Ala^{10}\text{-}NH_2 \quad (V)$$

in which D-Xxx is an amino acid group of the general formula (VI)



(VI)

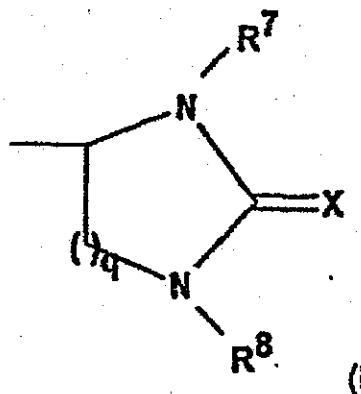
in which n is the number 3 or 4, R^4 is a group of the formula (II)



(II)

in which p is an integer from 1 to 4, R^5 is hydrogen or an alkyl group and R^6 is an unsubstituted or substituted aryl group or heteroaryl group,

or R^4 is a ring of the general formula (III)



(III)

in which q is the number 1 or 2, R^7 is a hydrogen atom or an alkyl group, R^8 is a hydrogen atom or an alkyl group and X is an oxygen or sulphur atom, and its salts with pharmaceutically acceptable acids,

and the DE 19911771 compounds of the following general formula I

$A\text{-}Xxx^1\text{-}Xxx^2\text{-}Xxx^3\text{-}Xxx^4\text{-}Xxx^5\text{-}Xxx^6\text{-}Xxx^7\text{-}Xxx^8\text{-}Xxx^9\text{-}Xxx^{10}\text{-}NH_2$ (I)

in which

A is an acetyl or a 3-(4-fluorophenyl)propionyl group,

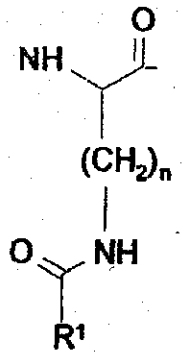
Xxx^1 is D-Nal(1) or D-Nal(2),

$Xxx^2\text{-}Xxx^3$ is D-Cpa-D-Pal(3) or a single bond,

Xxx^4 is Ser,

Xxx^5 is N-Me-Tyr,

Xxx^6 is D-Cit, D-Hci or a D-amino acid group of the general formula (II)

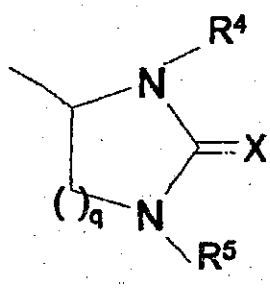


(II)

in which n is the number 3 or 4, where R^1 is a group having the general formula III

$$-(CH_2)_p-CO-NR^2R^3 \qquad (III)$$

where p is an integer from 1 to 4, R^2 is hydrogen or an alkyl group and R^3 is an unsubstituted or substituted aryl group or heteroaryl group, or R^1 is a 3-amino-1,2,4-triazole-5-carbonyl group or R^1 is a ring of the general formula (IV)



(IV)

in which q is the number 1 or 2, R^4 is a hydrogen atom or an alkyl group, R^5 is a hydrogen atom or an alkyl group and X is an oxygen or sulphur atom,
Xxx^7 is Leu or Nle,
Xxx^8 is Arg or Lys(iPr),
Xxx^9 is Pro and
Xxx^{10} is Ala or Sar,
and their salts with pharmaceutically acceptable acids.

Further peptides are abarelix, azaline B, detirelix, ramorelix (Stoeckemann and Sandow, J. Cancer Res. Clin. Oncol. 1993, 119, 457) and RS-68439. The structures of the peptides mentioned are found in: Behre et al., *GnRH antagonists: an overview*, Proceedings of the 2nd World Conference on Ovulation Induction, The Parthenon Publishing Group Ltd.; Kutscher et al., Angew. Chem. 1997, 109, 2240.

The acid addition salts of the peptides employed as starting materials are preferably easily soluble salts such as the acetates, hydrochlorides or sulphates.

According to the novel process, the starting peptide salt is completely or partly dissolved in a diluent or suspended therein. A diluent is then added. Solvent and diluent can be identical or different. Possible solvents and diluents are, for example: water, ethanol, methanol, propanol, isopropanol, butanol, acetone, dimethyl ketone, methyl ethyl ketone, dimethylacetamide, dimethylformamide, N-methylpyrollidone, acetonitrile, pentane, hexane, heptane and mixtures thereof. Ethanol, isopropanol or acetone are preferred. A water content of 1 to 60%, preferably of 5 to 50%, is likewise to be preferred.

The mixed bed ion exchanger, i.e. a mixture of an acidic and a basic ion exchanger, is added to the solution or suspension of the starting peptide salt. A possible ion exchanger is, for example, Amberlite®.

The amount of ion exchanger depends on the number of basic groups per peptide. The amount is determined by addition until a constant pH is obtained. For example, 10 g of Amberlite MB-3 are needed for 1 g of cetrorelix.

The pHs of the base solutions during the preparation of the bases is adjusted to 7.5 to 13, depending on the

active compound salt employed, in particular in the case of peptide salts with amino acids having a basic reaction, but in particular in the case of salts of LHRH antagonists (for example cetrorelix, D-63153, abarelix, ganirelix, ramorelix which can be present, for example, as acetate), depending on the active compound used.

The temperature should not exceed 25-30°C in order to avoid breakdown of the peptide. The reaction time for the preparation of the free base customarily takes place within a few minutes, for example 20 min starting from cetrorelix acetate. It can also be longer, for example approximately 1 h, starting from cetrorelix embonate. After achieving a constant pH, the reaction should be discontinued, since decomposition products, for example, can form on account of the basicity of the solutions.

The ion exchanger is then removed from the reaction mixture. Removal can be carried out by screening, filtering off, centrifuging or column filtration.

The clear to turbid solution of the free peptide base, which is unstable, should be reacted as rapidly as possible with the acid with formation of the desired acid addition salt. The acid can be added as a solid substance or in solution or as a suspension. In exactly the same way, the solution of the free peptide base can take place to the acid.

The reaction times are in the range from a few minutes to a few hours. For example, for the formation of cetrorelix embonate the reaction time is 1.5 h.

The reaction solution, which is customarily clear, is then sterile-filtered. The solvent can then be removed with obtainment of the pure peptide salt. Alternatively, before the removal of the solvent

excipients, additives or vehicles can be added to the solution. The addition of the excipients can take place as a solid before sterile filtration or after sterile filtration as a sterile-filtered solution.

Suitable excipients are, for example, mannitol, sorbitol, xylitol or soluble starch.

According to the invention, the following salts can be prepared by addition of the appropriate acid: acetate, adipate, ascorbate, alginate, benzoate, benzenesulphonate, bromide, carbonate, citrate, chloride, dibutylphosphate, dihydrogencitrate, dioctylphosphate, dihexadecylphosphate, fumarate, gluconate, glucuronate, glutamate, hydrogencarbonate, hydrogentartrate, hydrochloride, hydrogencitrate, iodide, lactate, alpha-lipoic acid, malate, maleate, malonate, pamoate, (embonate), palmitate, phosphate, salicylate, stearate, succinate, sulphate, tartrate, tannate, oleate or octylphosphate.

Example:

46.47 g of D-20761 were added in portions to 1193 g of water and dissolved with stirring. (= solution 1). Solution 1 was then diluted with 3261 g of 96% ethanol with stirring. (= solution 2). Solution 2 was filtered through a glass fibre prefilter after dilution and the filtrate was mixed with 390 g of Amberlite MB3 (mixed bed ion exchanger formed of strongly acidic cations and anion exchangers) (= mixture 1) with stirring.
316.8 g of mannitol was dissolved in 1267 g of water with stirring. (= solution 3).
After stirring for 15 min, the pH of the supernatant solution of mixture 1 was measured, and after stirring for a further 5 minutes the pH was measured again. The solution was then separated from the Amberlite MB3 through a fine-mesh sieve after a pH of 12.5 had been reached. (= solution 4).

4162 g of the solution 4 was mixed with 5.34 g of embonic acid with stirring. This mixture was vigorously stirred for a further 1.5 h and the somewhat turbid solution was then filtered through a glass fiber prefilter. In this solution was a pH of 8.4 (= solution 5). The measured pHs were measured with a ground-glass electrode using a viscous electrolyte liquid. The pHs were only to be regarded as relative values, since the solutions or suspensions measured contain ethanol and thus indicated an apparently higher values.

3333 g of the solution 5 were sterile-filtered in the reaction apparatus, which was adjusted to RT, and 528 g of the solution 3 were sterile-filtered to give the solution 5, which was adjusted to RT, with stirring. (= solution 6).

Solution 6 was warmed to 40°C and the ethanol/water mixture was then distilled off down to <= 1931 g in vacuo. (= suspension 1). The cetrorelix embonate suspension 1 was cooled to RT and diluted to 3000 g with sterile-filtered water for injection with stirring. (= suspension 2). 3.0 g each of the ready-to-use suspension 2, adjusted to RT, were then dispensed into 10 ml injection vials, provided with freeze-drying stoppers and transferred to the freeze-drying unit.

The injection vials were frozen in a freeze-drying unit at a plate temperature of -40°C. Drying was carried out by means of a drying programme at a plate temperature increasing from -40°C to 20°C. The FD unit with was flooded with sterile-filtered nitrogen, the injection vials in the unit were sealed and the crimp caps were then put on and rolled.

After the freeze drying, the sealed injection vials were sterilized by gamma radiation at 12 kGy (min) - 15 kGy. The latter is optional. 1 140.0(7) mg injection vial contains 34.07 mg of cetrorelix embonate corresponding to 30 mg of cetrorelix.

and 106 mg of mannitol. For reconstitution, 2 ml of water for injection are used. The suspension obtained can be administered by i.m. or s.c. administration.

Biological action:

The cetrorelix embonate (2:1) lyophilisate (30 mg) obtained according to example 1 is resuspended in 2 ml of water for injection and can then be administered parenterally, preferably subcutaneously (s.c.) or intramuscularly (i.m.).

In the case of s.c. administration, a bioavailability of about 30-50% (100% = intravenously administered cetrolix acetate) is found for the cetrorelix embonate (2:1). A particular advantage of cetrorelix embonate (2:1) lyophilisate is the small or no 'burst effect' in the patient. The duration of action is dose-dependent and is 2-8 weeks or longer at a dose of 30-150 mg. The cetrorelix embonate (2:1) lyophilisate according to the invention has already been investigated on humans in clinical phase 1.

Fig. 1 shows the course of the cetrorelix plasma concentration as a function of time (in hours) beginning from the i.m. administration of 60 mg of cetrorelix embonate (2:1) lyophilisate according to example 1 in humans. No burst effect (about 100 ng/ml) is determinable. Duration of action more than 700 hours. From 150 h after administration constant plasma level of about 2 ng/ml. The bioavailability was about 40%.

The areas of application of the peptide salts according to the invention are, for example, BPH, myoma and endometriosis.

Patent Claims:

1. Process for the production of a pharmaceutical preparation for parenteral administration to mammals, characterized in that an easily soluble acid addition salt of a basic peptidic LHRH antagonist is reacted in the presence of a suitable diluent with a mixed bed ion exchanger or a mixture of an acidic and a basic ion exchanger with formation of the free basic peptide, the ion exchanger is subsequently separated off and the free basic peptide is then reacted with an inorganic or organic acid with formation of the desired poorly soluble acid addition salt of the peptide, suitable pharmaceutical excipients, vehicles and/or bulking agents are then added and the diluent is subsequently removed.

2. Process according to claim 1, characterized in that a salt of cetrorelix, teverelix, abarelix, ganirelix, azaline B, antide, A-75998, detirelix, ramorelix or RS-68439 is employed as the starting peptide salt.

3. Process according to claim 1 or 2, characterized in that the acid is embonic acid, stearic acid or salicylic acid.

4. Process according to one of claims 1 to 3, characterized in that the molar ratio of cetrorelix to embonic acid is 2:1.

5. Process according to one of claims 1 to 4, characterized in that the diluent is removed by freeze drying.