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(54) TRH L-TARTRATE CRYSTALS

(71) We, TAKEDA YAKUHIN 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 present invention relates to a crystalline derivative of L-pyroglutamyl-L-histidyl-L-proline amide (thyrotropin releasing hormone; hereafter sometimes referred to briefly as "TRH").

While TRH has heretofore been prepared by various methods, the final products are invariably amorphous powders such as solvent-free and/or lyophilized products and no crystalline products have been available as yet. These non-crystalline or amorphous powders are generally low inpurity, suffer a large variation in purity between product batches and are hygroscopic and unstable. Therefore, attempts have been made, in vain, to produce crystals in the hope of obtaining high-purity, homogeneous and non-hygroscopic products.

We have, therefore, sought to provide a novel TRH derivative the crystals of which are stable against moisture, and an industrially feasible method for producing these crystals.

Accordingly, the present invention provides a crystal of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent of crystallisation.

The TRH L-tartrate crystals of the present invention can be obtained by causing TRH L-tartrate to separate as crystals from a solution comprising TRH, L-tartaric acid and a solvent to be included in the crystals as a solvent of crystallization.

TRH as employed as the starting material according to this invention may be in various forms, e.g. the free compound or the corresponding acetate or hydrochloride and may be of various purity grades.

When use is made of the above-mentioned acetate or hydrochloride, for instance, it may before use be converted to the free base by means of an ion-exchange resin (for example, a basic anion-exchange resin, e.g. Amberlite (Trade Mark) IRA-400, Amberlite IRA-410, Amberlite IR-4B or Dowex (Trade Mark) 1, Dowex 2, Dowex 3, etc.), if so desired.

The tartaric acid which is essentially L-tartaric acid can be used not only in the free form but also as a salt (e.g. a calcium, potassium, sodium or other salt). When TRH is used in the form of a salt with an acid, it is advantageous to employ L-tartaric acid in the above-mentioned salt form.

While the proportion of L-tartaric acid is not particularly critical for the purposes of this invention, it is preferably used in amounts of not less than 0.5 mole and, most preferably, from 0.8 to 1.4 moles per mole of TRH.

The solvent may be any solvent which can be included in the crystal of TRH L-tartrate as a solvent of crystallization, for instance, water, methanol or acetonitrile.

To precipitate crystals of TRH L-tartrate from a solution comprising TRH, L-tartaric acid and a solvent system containing a solvent to be included in the crystals as a solvent of crystallization, crystallization procedures which are known *per se* can be successfully employed. For example, use can be made of a method in which a solvent miscible with the solution in which TRH tartrate is insoluble at all or only slightly soluble e.g. ethanol, *n*-propanol, isopropanol, acetone, dioxane or the like, is added to the above-mentioned solution so as to depress the solubility of TRH tartrate

in the mixed solvent; a method which comprises cooling a saturated solution to cause the solute to crystallize by taking advantage of the variable solubility of the solute at different temperatures of the solvent; and a method which comprises the removal of the solvent from the solution by distillation. The crystals separating out from the solution can be collected by recovery procedures which are known *per se*, examples of which are filtration and centrifuging.

The TRH L-tartrate crystals thus obtained by the method mentioned above are of excellent homogeneity, each crystal being formed as a molecular compound comprising equimolar proportions of TRH and L-tartaric acid and further comprising an equimolar proportion of the solvent of crystallization.

Moreover, the solvent of crystallization in the crystal can be replaced with another solvent by contacting a TRH L-tartrate crystal including a certain solvent as a solvent of crystallization with another solvent. Thus, a TRH L-tartrate crystal including water as the solvent of crystallization (hereafter referred to sometimes as TRH L-tartrate-water) can be converted to a TRH L-tartrate crystal including methanol or acetonitrile as the solvent of crystallization (hereafter referred to sometimes as TRH L-tartrate-methanol or TRH L-tartrate-acetonitrile, respectively) and the latter crystal can be harvested. Conversely, TRH L-tartrate-methanol or TRH L-tartrate-acetonitrile can be converted to a crystal of TRH L-tartrate including another solvent of crystallization and the latter crystal can be collected.

Thus, for example, TRH L-tartrate-methanol crystals can easily be obtained by adding more than 3 times and preferably 5 to 15 times the weight of TRH L-tartrate-water crystals of methanol and collecting the resulting transformed crystals by a recovery procedure as set forth above. Similarly, TRH L-tartrate-methanol crystals can be transformed to TRH L-tartrate-water crystals and the latter crystals can be collected. Thus, TRH L-tartrate-water crystals can be obtained by adding water to TRH L-tartrate-methanol crystals in a proportion corresponding to not less than 0.05 and preferably 0.1 to 5 times the weight of the crystals and collecting the transformed crystals according to the recovery procedure described above.

The crystals thus obtained are very pure and extremely stable against moisture. For example, we found that when the crystal had been maintained at 60°C and at 60% relative humidity for 4 months it was not changed at all. TRH L-tartrate or TRH L-tartrate-water is less toxic to human beings or animals and is useful as medicine for the same purposes as those of TRH, for instance, as an anti-depressor or as an agent for the diagnosis of the hypophysis function.

Thus, the invention also provides a pharmaceutical composition comprising crystals of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent of crystallisation together with a non-toxic pharmaceutically acceptable carrier or diluent therefor.

The dosage, preparation and administration means may be determined by reference to those of TRH itself. Further, a pure preparation of TRH can easily be obtained by separating out TRH from a solution in which TRH L-tartrate crystals are dissolved by means of an ion exchange resin. Therefore, the crystallization of TRH L-tartrate can advantageously be applied to the purification of TRH.

Figure 1 of the accompanying drawings shows a photograph of TRH L-tartrate-water crystals of the present invention magnified to 124 diameters with a microscope.

Figure 2 of the accompanying drawings shows an X-ray diffraction pattern of TRH L-tartrate-water measured by the powder method. The abscissa (2θ) is the angle of diffraction and the significant lattice spacings are as follows:

2.74	angstrom	(middle)
3.18	angstrom	(middle)
3.39	angstrom	(weak)
3.56	angstrom	(middle)
3.60	angstrom	(middle)
3.87	angstrom	(middle)
4.17	angstrom	(middle)
4.37	angstrom	(strong)
5.09	angstrom	(middle)
5.63	angstrom	(middle)
6.06	angstrom	(middle)
6.41	angstrom	(strong)
7.43	angstrom	(weak)

Further, the nuclear magnetic resonance spectrum of the TRH L-tartrate-water crystals is measured, and the significant δ values are as follows:

(D_2O)δ: 8.70(s, 1, Im-2H-His), 7.43(s, 1, Im-4H-His), 5.10(t, 1, αH-His), 4.60(s, 2, α- and β-CH-tart), 4.54—4.34(m, 2, αH-Pro and αH-pGlu), 3.82—3.62(m, 2, δδ′-CH_2-Pro), 3.32(q, 2, ββ′-CH_2His), 2.60—1.92(m, 8, ββ′+γγ′-CH_2-pGlu and ββ′+γγ′-CH_2-Pro).

The symbols used in the nuclear magnetic resonance spectra of Figures 2, 4 and 5 are explained as follows:

8.70 (s, 1, Im-2H-His) for example means
8.70: δ value
δ: splitting pattern (singlet) cf. d: doublet, t: triplet, q: quartet, m: multiplet
1: number of protons
Im-2H-His: position of this proton (at 2-position of imidazole ring of histidine).

Figure 3 of the accompanying drawings shows a photograph of the TRH L-tartrate-methanol crystals of the present invention magnified to 100 diameters with a microscope.

Figure 4 of the accompanying drawings shows an X-ray diffraction pattern of TRH L-tartaric-methanol measured by the powder method, and the significant lattice spacings are as follows:

2.82	angstrom	(weak)
2.96	angstrom	(weak)
3.45	angstrom	(middle)
3.60	angstrom	(middle)
3.79	angstrom	(weak)
3.90	angstrom	(weak)
4.17	angstrom	(middle)
4.35	angstrom	(middle)
4.42	angstrom	(strong)
4.48	angstrom	(middle)
5.10	angstrom	(middle)
6.07	angstrom	(middle)
6.42	angstrom	(strong)

Further, the nuclear magnetic resonance spectrum of theTRH L-tartrate-methanol crystals is measured, and the significant δ values are as follows:

(D_2O)δ: 8.70(s, 1, Im-2H-His), 7.43(s, 1, Im-4H-His), 5.10(t, 1, αH-His), 4.60(s, 2, α- and β-CH-tart), 4.54—4.34(m, 2, αH-Pro and αH-pGlu), 3.82—3.62(m, 2,δδ′-CH_2-Pro), 3.4(s, 3, CH_3OH), 3.32(q, 2, ββ′-CH_2-His), 2.60—1.92(m, 8, ββ′+γγ′-CH_2-pGlu and ββ′+γγ′-CH_2-Pro).

Figure 5 of the accompanying drawings shows an X-ray diffraction pattern of TRH L-tartrate-acetonitrile measured by the powder method, and the significant lattice spacings are as follows:

2.83	angstrom	(weak)
3.04	angstrom	(weak)
3.24	angstrom	(middle)
3.47	angstrom	(middle)
3.59	angstrom	(middle)
3.62	angstrom	(middle)
3.93	angstrom	(weak)
4.19	angstrom	(middle)
4.40	angstrom	(middle)
4.46	angstrom	(strong)
5.13	angstrom	(middle)
6.11	angstrom	(weak)
6.51	angstrom	(middle)

Further, the nuclear magnetic resonance spectrum of the TRH L-tartrate-acetonitrile crystals is measured and the significant δ values are as follows:

$(D_2O)\delta$: 8.70(s, 1, Im-2H-His), 7.43(s, 1, Im-4H-His), 5.10(t, 1, αH-His), 4.60(s, 2, α- and β-CH-tart), 4.54—4.34(m, 2, αH-Pro and αH-pGlu), 3.82—3.62(m, 2, $\delta\delta'$-CH_2-Pro), 3.32(q, 2, $\beta\beta'$-CH_2-His), 2.60—1.92(m, 8, $\beta\beta'+\gamma\gamma$-CH_2-pGlu and $\beta\beta'+\gamma\gamma'$-$CH_2$-Pro), 2.1(s, 3, CH_3CN).

For a further explanation of the present invention the following examples are given which the word "part(s)" is based on weight unless otherwise noted, and the relation between "part" and "volume part" corresponds to that between gram and milliliter.

Example 1

In 50 volume parts of water are dissolved 19 parts of TRH monohydrate and 7.5 parts of L-tartaric acid, and, while the solution is warmed to 70°C, ethanol is added in portions. Immediately after a slight turbidity is observed, the temperature is gradually brought down to room temperature, at which temperature the solution is allowed to stand for 3 days. The solution is then cooled to 10°C and allowed to stand for 2 days to allow crystallization to be completed. The crystals thus obtained are collected by filtration, washed with hot ethanol and dried under reduced pressure at 50°C for 1 hour and at 80°C for 5 hours. The procedure described yields 18 parts of TRH L-tartrate-water as crystals melting at 127°C—130°C.

$[\alpha]_D^{24}=-49.90°$ (c=1.05 in water)

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot H_2O$

Calcd.	C, 45.28;	H, 5.70;	N, 15.84
Found	C, 45.21;	H, 5.70;	N, 15.81

Reference Example

Crystals of TRH L-tartrate-water obtained above are dried under reduced pressure (0.5 mmHg) at 80—90°C for 8 hours, whereby crystals of TRH L-tartrate-water melting at 150—155°C (with decomposition) are obtained.

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot \frac{1}{2}H_2O$

Calcd.	C, 46.06;	H, 5.60;	N, 14.46
Found	C, 45.97;	H, 5.63;	N, 14.33

Example 2

In 30 volume parts of water are dissolved, under heating, 3.8 parts of powdery TRH and 1.5 parts of L-tartaric acid. After the solution has been cooled to room temperature, a few pieces of crystals obtained according to Example 1 are added as seeds and the water is evaporated off gently in a desiccator, whereupon the entire residue is obtained as crystals.

Following the addition of a relatively small amount of cold ethanol, the crystals are collected by filtration, washed with ethanol and dried. The procedure described yields 4.6 parts of TRH L-tartrate-water as crystals melting at 127°C—130°C.

$[\alpha]_D^{24}=-49.90°$ (c=1.05 in water).

Example 3

In 25 volume parts of methanol are dissolved, under heating, 1.9 parts of crude TRH (including approximately 10% of impurity) and 0.75 part of L-tartaric acid, and the solution is allowed to stand at room temperature for 7 days. The resulting crystals are collected by filtration and dried under reduced pressure at 50°C for 5 hours. The procedure described yields 1.8 parts of crystals of a molecular compound of TRH L-tartrate including one molecule of methanol as crystals melting at 137°C—140°C.

$[\alpha]_D^{25}=-46.97°$ (c=1.01 in water)

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot CH_3OH$

Calcd.	C, 46.32;	H, 5.92;	N, 15.44
Found	C, 46.19;	H, 5.91;	N, 15.29

Example 4

In 60 volume parts of methanol are dissolved, under heating, 3.6 parts of dry TRH powder and 1.5 parts of L-tartaric acid.

A total of 40 volume parts of acetonitrile is added in small portions to the above solution and the mixture is allowed to stand at room temperature for 3 days and then in a refrigerator for 2 days. The resulting crystals are collected by filtration and dried under reduced pressure at 50°C for 8 hours. The procedure yields 3.8 parts TRH L-tartrate-methanol as crystals: melting at 137°C—140°C.

$[\alpha]_D^{24} = -50.25°$ (c=1.015 in water)

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot CH_3OH$

Calcd.	C, 46.32;	H, 5.92;	N, 15.44
Found	C, 46.21;	H, 5.90;	N, 15.31

Example 5

In 50 volume parts of water are dissolved 19.0 parts of TRH · H_2O and 7.5 parts of L-tartaric acid, followed by the addition of 700 volume parts of acetone. The resulting oil is rubbed with a glass rod and the crystals formed are recovered by filtration, washed with hot ethanol and dried under reduced pressure. The procedure yields 24.5 parts of TRH L-tartrate-water as crystals melting at 127°C—129°C.

$[\alpha]_D^{25} = -49.45°$ (c=1.0 in water)

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot H_2O$

Calcd.	C, 45.29;	H, 5.70;	N, 15.84
Found	C, 45.04;	H, 5.56;	N, 15.88

Example 6

In 25 volume parts of water are dissolved 19.0 parts of TRH · H_2O and 7.5 parts of L-tartaric acid. To the solution are added 400 volume parts of dioxane and the resulting oil is crystallized by rubbing with a glass rod.

The crystals are recovered by filtration, washed with hot ethanol and dried under reduced pressure. The procedure yields 22.4 parts of TRH L-tartrate-water as crystals melting at 126°C—128°C.

$[\alpha]_D^{25} = -48.90°$ (c=1.0 in water)

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot H_2O$

Calcd.	C, 45.28;	H, 5.70;	N, 15.84
Found	C, 45.00;	H, 5.82;	N, 15.62

Example 7

In 6 volume parts of 1.7 N hydrochloric acid are dissolved 3.99 parts of TRH hydrochloride and 2.62 parts of calcium L-tartrate tetrahydrate ($CaC_4H_4O_6 \cdot 4H_2O$), followed by the addition of 20 volume parts of ethanol. This solution is seeded with TRH L-tartrate-water crystals and a further amount of ethanol is added gradually to make a total of 40 volume parts of added ethanol. The resulting crystals are recovered by filtration and treated in the same manner as in Example 1. The procedure yields 3.81 parts of TRH L-tartrate-water as crystals melting at 127°C—130°C.

$[\alpha]_D^{25} = -49.90°$ (c=1.05 in water).

Example 8

In 80 volume parts of acetic acid are dissolved 19 parts of TRH monohydrate and 7.5 parts of L-tartaric acid, and while the solution is warmed to 50°C, 240 volume parts of acetonitrile are added in portions. The temperature is gradually brought down to room temperature, at which temperature the solution is allowed to stand overnight. The resulting crystals are collected by filtration, washed with acetonitrile and dried under reduced pressure. The procedure yields 25.7 parts of TRH L-tartrate-acetonitrile as crystals melting at 145°C—148°C.

$[\alpha]_D^{22} = -47.1°$ (c=1.0 in water).

Elemental analysis, for $C_{16}H_{22}O_4N_6 \cdot C_4H_6O_6 \cdot CH_3CN$

Calcd.	C, 47.74;	H, 5.65;	N, 17.72
Found	C, 47.79;	H, 5.77;	N, 17.58

WHAT WE CLAIM IS:—

1. A crystal of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent of crystallization.

2. A crystal as claimed in claim 1, wherein the solvent of crystallization is water.

3. A crystal as claimed in claim 1, wherein the solvent of crystallization is methanol.

4. A crystal as claimed in claim 1, wherein the solvent of crystallization is acetonitrile.

5. A method of producing crystals of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent of crystallization which comprises precipitating L-pyroglutamyl-L-histidyl-L-prolineamide L-tartrate as crystals from a solution comprising L-pyroglutamyl-L-histidyl-L-proline amide, L-tartaric acid and a solvent to be included in the crystal as a solvent of crystallization.

6. A method as in claim 5, wherein the solvent consists of one or more of water, methanol and acetonitrile.

7. A method as claimed in claim 6, wherein the solvent is water.

8. A method as claimed in claim 6, wherein the solvent is methanol.

9. A method as claimed in claim 6, wherein the solvent is acetonitrile.

10. A method as claimed in claim 5, substantially as herein described with reference to any of the specific Examples.

11. Crystals of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent when made by a method as claimed in any of claims 5—10.

12. A pharmaceutical composition comprising crystals of L-pyroglutamyl-L-histidyl-L-proline amide L-tartrate including an equimolar amount of a solvent of crystallization together with a non-toxic pharmaceutically acceptable carrier or diluent therefor.

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