Description

DIPEPTIDE AND PHARMACEUTICAL COMPOSITION CONTAINING SAME

Technical Field

[0001]

The present invention relates to a dipeptide and a pharmaceutical or food product composition containing the same.

Background Art

[0002]

Fatigue generally has a feeling of tiredness and malaise as cardinal symptoms but consequently causes reduced motor ability, disturbed sleep, loss of motivation, and the like. In the case of short-term fatigue, these symptoms are recovered by rest, sleep, nutritional support and the like, whereas in the case of chronic fatigue, long-term overall feeling of tiredness, malaise, mild fever, and the like are hardly recovered.

[0003]

To counteract these fatigues, amino acid compositions (Patent Literature 1), L-carnitine (Patent Literature 2), ascorbic acids (Patent Literature 3), and the like are known to be useful. Additionally, a liver hydrolysate is documented to have an AMPK activation action and an antifatigue effect (Patent Literature 4).

Citation List

Patent Literature

[0004]

Patent Literature 1: JP-A-9-124473

Patent Literature 2: JP-A-2001-046021

Patent Literature 3: JP-A-6-327435

Patent Literature 4: WO 2015/022927

Summary of Invention

Technical Problem

[0005]

A liver hydrolysate is considered to contain abundant amino acids and peptides, however, the actual active component has not been revealed.

Thus, an object of the present invention is to provide a novel component having an action of preventing or improving fatigue.

Solution to Problem

[0006]

The present inventors hence studied to develop a novel agent of preventing or improving fatigue, investigated for indigestible peptides which enter the blood by administering a liver hydrolysate to an animal and evaluated pharmacological effects by fractionating peptides of a liver hydrolysate using various columns, and, as a result, found dipeptides derived from specific D-amino acids, not from an L-amino acid, and also found

that the dipeptides have an action of preventing or improving fatigue whereby the present invention was accomplished.

[0007]

More specifically, the present invention provides the following [1] to [9].

[0008]

[1] A dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[2] The dipeptide according to [1], wherein the dipeptide is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[3] A pharmaceutical composition comprising the dipeptide according to [1] or [2].

[4] The pharmaceutical composition according to [3], which is an agent for preventing or improving fatigue.

[5] A food product composition comprising the dipeptide according to [1] or [2].

[6] The food product composition according to [5], which is a food product composition for preventing or improving fatigue.

[7] Use of the dipeptide according to [1] or [2] for producing an agent for preventing or improving fatigue or a food product for preventing or improving fatigue.

[8] The dipeptide according to [1] or [2] for use in prevention or improvement of fatigue.

[9] A method for preventing or improving fatigue, comprising administering an effective amount of the dipeptide according to [1] or [2].

Advantageous Effects of Invention

[0009]

The dipeptides of the present invention are indigestible as they are derived from D-amino acids, stay in blood for an extended period of time and have an action of preventing or improving fatigue thereby being useful as pharmaceutical and food product compositions for preventing or improving fatigue.

Brief Description of Drawings

[0010]

[Figure 1] Figure 1 shows an antifatigue effect of a hydrophobic peptide fraction.

[Figure 2] Figure 2 shows an antifatigue effect of a hydrophobic pyroglutamyl peptide fraction.

[Figure 3] Figure 3 shows an antifatigue effect of a hydrophilic peptide fraction.

[Figure 4] Figure 4 shows an antifatigue effect of a hydrophilic pyroglutamyl peptide fraction.

[Figure 5] Figure 5 shows an antifatigue effect of Asp-Leu (Dα).

[Figure 6] Figure 6 shows an antifatigue effect of Asp-Leu (Dβ).

[Figure 7] Figure 7 shows an antifatigue effect of Asp-Val (Dα).

[Figure 8] Figure 8 shows an antifatigue effect of Asp-Val (Dβ).

[Figure 9] Figure 9 shows an antifatigue effect of Asp-Phe (Dα).

[Figure 10] Figure 10 shows an antifatigue effect of Asp-Phe (Dβ).

Description of Embodiments

[0011]

The dipeptide of the present invention is a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and

(D)Asp-(L)Phe, among them, a dipeptide selected from the group consisting of D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe is preferable from the viewpoint of ability to enter the blood when orally administered, action sustainability and antifatigue effect, and (D)Asp-(D)Leu and (D)Asp-(L)Leu are further preferable.

(D) as represented herein means that an amino acid is the D-amino acid. (L) as represented means that an amino acid is the L-amino acid.

Further, among the α-amino acid and the β-amino acid of the above (D)- or (L)-dipeptides, the β-amino acid is more preferable.

[0012]

The dipeptide of the present invention can be produced using a (D)-amino acid as a starting material by a typical liquid-phase peptide synthesis method or solid-phase peptide synthesis method. For example, an amino acid in which the functional groups other than the α-amino group are protected and an amino acid in which the functional groups other than the carboxyl group are protected or an amino acid in which the carboxyl group is activated and in which the functional groups other than the carboxyl group are protected are condensed, and subsequently the protecting groups are deprotected to produce the dipeptide.

Examples of the protecting group for the amino group of an amino acid herein include a benzyloxycarbonyl group, a tert-butoxycarbonyl group, a fluorenylmethoxycarbonyl group, and the like. Examples of the protecting group for the carboxyl group include a tert-butyl group, a benzyl group, and the like. The condensation reaction can employ a method of using N,N'-dicyclohexylcarbodiimide, dicyclohexylurea, or other condensation agents, an active ester method by use of, for example, nitrophenol and N-hydroxysuccinimide, a mixed acid anhydride method, or the like.

The protecting groups are removed after completing the condensation reaction and, in the case of the solid-phase method, the link between the C-terminal end of the peptide and a resin is further cleaved. Further, the peptide of the present invention is purified in accordance with a typical method.

[0013]

The dipeptide of the present invention can be an acid addition salt or a base salt. Examples of the acid addition salt include a salt of an inorganic acid such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, hydrobromic acid, and perchloric acid, and a salt of an organic acid such as citric acid, succinic acid, maleic acid, fumaric acid, malic acid, tartaric acid, p-toluenesulfonic acid, benzenesulfonic acid, methanesulfonic acid, and trifluoroacetic acid. Examples of the base salt include a salt of an alkali metal such

as sodium, potassium, and lithium and a salt of an alkaline earth metal such as calcium and magnesium.

[0014]

The peptide of the present invention can be a solvate. Examples of the solvate include a solvate of water (in the case of a hydrate), methanol, ethanol, isopropanol, and the like.

[0015]

The peptide of the present invention is indigestible with good ability to enter blood after orally administered and good action sustainability and has an action of preventing or improving fatigue. Examples of the action of preventing or improving fatigue herein include an action of sustaining motor function, an action of improving motor function, an antifatigue action, an antistress action, an action of alleviating a feeling of tiredness, an action of preventing chronic fatigue, a fatigue recovery action, an action of preventing or improving palpitation, an action of preventing or improving restoration, an action of preventing or improving shortness of breath, an action of preventing or improving muscle ache, and the like. Thus, a composition comprising the dipeptide of the present invention is useful as a pharmaceutical composition and a food product composition.

[0016]

The pharmaceutical composition of the present invention can be administered by oral administration,

transdermal administration, enteral administration, intravenous administration or the like, and oral administration is more preferable. Examples of the formulation for oral administration include a liquid preparation, a tablet, a powder, a fine granule, a granule, a capsule, and the like, with a liquid preparation and a tablet being preferable, and a liquid preparation being more preferable.

[0017]

These oral administration formulations can be prepared by using an excipient such as lactose, mannitol, cornstarch, or crystalline cellulose, a binder such as a cellulose derivative, gum arabic, or gelatin, a disintegrating agent such as calcium carboxymethylcellulose, a lubricant such as talc or magnesium stearate, a solubilizing agent such as a nonionic surfactant, a flavoring agent, a sweetener, a stabilizer, a pH adjuster, water, ethanol, propylene glycol, glycerin, or the like. Additionally, a coating agent such as hydroxymethylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, cellulose acetate phthalate, or a methacrylate copolymer may also be used.

[0018]

Further, the pharmaceutical composition of the present invention can also contain other active components. Examples of the other active components include vitamin B_1 compounds such as thiamine, thiamine

nitrate, thiamine hydrochloride, fursultiamine, bisbentiamine, benfotiamine, thiamine disulfide, dicethiamine, thiamine propyl disulfide, and a derivative thereof, vitamin B_2 compounds such as riboflavin and a derivative thereof and a salt thereof, vitamin B_3 compounds such as niacin, nicotinic acid, nicotinamide, and a derivative thereof and a salt thereof, vitamin B_5 compounds such as panthenol, pantothenic acid, and a derivative thereof and a salt thereof, vitamin B_6 compounds such as pyridoxine and a derivative thereof and a salt thereof, vitamin B_{12} compounds such as cyanocobalamin and a derivative thereof and a salt thereof, other vitamin compounds such as vitamin A, vitamin C, vitamin E, vitamin K, and vitamin P, diisopropylamine dichloroacetate, taurine, chondroitin sulfate, royal jelly, caffeine, turmeric, milk thistle, Japanese dandelion, dandelion, burdock, garlic, chrysanthemum, yarrow, common gardenia, sesame, notoginseng, asparagus, onion, chicory, common sage, artichoke, Chinese desert-thorn, plants of the family Fabaceae or the family Iridaceae, miyama-uzura (*Goodyera schlechtendaliana*), Erva-de-passarinho, Sete-Sangria, Japanese Mallotus, black tea, resveratrol, catechin compounds, berberine, rosemary, bean extract, metformin, and the like.

[0019]

Further, the composition of the present invention can also be used as, in addition to a pharmaceutical

product, a quasi-drug, a specific health food, and a functional food such as a sports drink, a drink for rehabilitation, and a pet food.

[0020]

A content of the dipeptide in the pharmaceutical composition or the food product composition of the present invention varies depending on the dosage form, and is typically preferably from 0.001 to 10 mass% and more preferably from 0.001 to 5 mass%. Additionally, a daily dose of the dipeptide in the pharmaceutical composition or the food product composition of the present invention is preferably from 10 mg to 1,000 mg, more preferably from 20 mg to 800 mg, and further preferably from 50 mg to 800 mg.

Example

[0021]

Subsequently, the present invention is described in detail in reference to examples but the present invention is not limited thereto.

[0022]

Example 1 (Fractionation of a liver hydrolysate)

(1) Fractionation of peptide and pyroglutamyl peptide

A strong cation exchange resin (AG50) was filled in an Econo Column (2.5 × 20 cm) and the resin was equilibrated with 10 mM HCl. In 20 mL of 10 mM HCl, 1 g of a liver hydrolysate (Sample A) was dissolved. The solution was added onto the resin and 20 mL of a flow-

through fraction was collected (flow-through fraction 1). Subsequently, 20 mL of 10 mM HCl was added onto the resin and 20 mL of a flow-through fraction was collected. This operation was repeated 19 times (flow-through fractions 2 to 20). The absorbance (230 nm) of the flow-through fractions 1 to 20 was measured to confirm the elution of the peptides.

Subsequently, 20 mL of a 50% ammonia solution was added onto the resin and 20 mL of an adsorbed fraction was collected. This operation was repeated 20 times (adsorbed fractions 1 to 20). The absorbance (230 nm) of the adsorbed fractions 1 to 20 was measured to confirm the elution of thepeptides.

The flow-through fractions (pyroglutamyl peptide fractions) and the adsorbed fractions (peptide fractions) were concentrated under reduced pressure using an evaporator.

[0023]

(2) Hydrophilic and hydrophobic fractionation

A solid-phase extraction column (Sep-Pak) was equilibrated with 10 mM HCl. The pyroglutamyl peptide fraction was allowed to pass through the column to elute and collect a flow-through fraction (hydrophilic pyroglutamyl peptide fraction). Subsequently, a 60% acetonitrile solution containing 10 mM HCl was allowed to pass through the column to elute and collect an adsorbed fraction (hydrophobic pyroglutamyl peptide fraction).

The peptide fraction was also subjected to these operations.

The obtained 4 fractions were lyophilized.

[0024]

Example 2

(1) Quantitative experiment of indigestible peptides in liver hydrolysate

1) In 1 mL of 50 mM Tris-HCl, 2.5 mg of a liver hydrolysate was dissolved.

2) Pancreatin (0.1 mg), leucine aminopeptidase (2.45 unit) and carboxypeptidase (7.7 unit) were added to 1) to cause an enzyme reaction (37°C, 24h).

3) The enzymes were removed by ultrafiltration (10K).

4) 3) was allowed to pass through a strong cation exchange resin (AG50) filled in a spin column to collect a flow-through fraction (pyroglutamyl peptide fraction).

5) By size exclusion HPLC (SEC Fr.35-44), 200 μL of 3) (peptide fraction) and 4) (pyroglutamyl peptide fraction) were fractionated and separated.

6) For the peptide fraction, SEC Fr.35-44 were solidified to dryness and derivatized with AccQ. For the pyroglutamyl peptide fraction, SEC Fr.35-44 were directly used.

7) The structure of indigestible peptide was determined by LC-MS/MS analysis.

Column: Inertsil ODS-3

Eluent: 0.1% formic acid and 80% acetonitrile containing 0.1% formic acid

Analysis method: for the peptide fraction, AccQ fragment (m/z = 171.1) was specifically detected (Precursor ion scan analysis), subsequently the structure was estimated by MS/MS analysis to synthesize a standard whereby identification and quantification are carried out by MRM analysis.

For the pyroglutamyl peptide fraction, peaks are detected by total scan ion analysis, subsequently the structure was estimated by MS/MS analysis to synthesize a standard whereby identification and quantification are carried out by MRM analysis.

[0025]

(2) Experiment on ability of peptide to enter the blood when a liver hydrolysate was administered to a rat

1) A single dose of an aqueous solution of a liver hydrolysate was administered to a Wister rat (6-week old, male) (10 g/60 kg).

2) Blood was collected from the abdominal vena cava under isoflurane anesthesia 30 min and 60 min after the administration, to thereby obtain plasma. Further, the digestive tract (duodenum to ileum) is removed and luminal contents are dissolved out with 10 mL of saline. Three times the amount of ethanol was added to the plasma and the luminal contents. (preserved at -20°C until analyzed).

3) The ethanol supernatant of plasma and luminal contents was solidified to dryness and derivatized with AccQ.

4) Identification and quantification of the indigestible peptides identified in (1) were carried out by MRM analysis.

The dipeptide concentrations in blood are shown in Table 1.

[0026]

[Table 1]

(pmol mL)

(min)	Asp-Leu (D α)	Asp-Val (D α)	Asp-Ile (D α)	Asp-Phe (D α)
0	2.03±0.94	ND	2.03±0.94	0.09±0.16
60	16.43±2.09**	8.84±0.85**	16.43±2.10**	2.08±0.09**
(min)	Asp-Leu (D β)	Asp-Val (D β)	Asp-Ile (D β)	Asp-Phe (D β)
0	3.41±0.50	ND	3.56±1.28	1.54±0.62
60	28.54±2.83**	16.31±3.27*	26.37±2.66**	17.67±3.25**

Mean (n = 3) ± SD *p<0.05, **p<0.01

Asp-Leu (Dα), Asp-Val (Dα): As Asp-Ile and Asp-Leu were not separated by LC, both are Asp-Ile + Asp-Leu (Dα).

[0027]

As a result, it was revealed that (D)Asp-(D)Val, (D)Asp-(D)Phe, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe had good ability to enter the blood when orally administered and sustained in blood for an extended period of time.

[0028]

Example 3 (Synthesis of dipeptide)

1) Reagents were added to a round-bottom flask in the following order to cause a reaction while stirring (4°C, overnight).

(i) H-Leu-OtBu·HCl

(ii) DMF

(iii) TEA

(iv) Boc-Asp(OtBu)-OH (Lα amino acid)

(v) HOBt

(vi) EDL·HCl

In the case of other isomers, the following protected amino acids are used.

Boc-D-Asp(OtBu)-OH (Dα amino acid)

Boc-Asp-OtBu (Lβ amino acid)

Boc-D-Asp-OtBu (Dβ amino acid)

2) DMF is removed using an evaporator.

3) The resultant is dissolved in ethyl acetate and moved to a separatory funnel.

4) Thereto, 5% sodium hydrogen carbonate is added, stirred and the aqueous layer is removed. (× 2)

5)Thereto, 10% citric acid is added, stirred and the aqueous layer is removed. (× 2)

6) Thereto, saturated saline is added, stirred and the aqueous layer is removed.

7) The ethyl acetate layer is collected and sodium hydrogen sulfate is added to dehydrate.

8) The ethyl acetate layer is collected by filtration and concentrated using an evaporator.

9) Petroleum ether is added thereto and precipitation caused is dried. (When not caused, proceed to 10))

10) 4M HCl/dioxane is added to the dried product and reacted wile stirring. (4°C, 24 to 48h)

11) 4M HCl/dioxane is removed using an evaporator.

12) Diethyl ether is added thereto, the obtained precipitation is ultrasonically crushed and washed, and the ether supernatant is removed by decantation. (× 3)

13) Diethyl ether is added thereto and allowed to stand (4°C, overnight).

14) Diethyl ether is added thereto, the obtained precipitation is ultrasonically crushed and washed, and the ether supernatant is removed by decantation. (× 3)

15) The precipitate is dried.

[0029]

Example 4

[Test method]

(1) Animal used

For experiments, ddY male mice having body weights of 28 to 32 g (26 g upon delivery) (Japan SLC, Inc.) were used and kept under the constant environment with a room temperature of 22 ± 2°C, a humidity of 55 ± 5% and a 12/12 hour-light/dark cycle (light period; 7:00 to 19:00, dark period 19:00 to 7:00) until subjected to experiments. A plastic cage (length 30 cm × width 20 cm × height 13 cm) was used while keeping and the mice were fed with pellets and tap water ad libitum when not subjected to the experiment.

(2) Drugs used and method of preparation

· The hydrophobic peptide fraction, hydrophobic pyroglutamyl peptide fraction, hydrophilic peptide fraction, and hydrophilic pyroglutamyl peptide fraction

were dissolved in saline and were intraperitoneally (i.p.) administered in an amount of 0.1 mL per 10 g of a body weight. For administration scheme, the mice were i.p. administered with saline, the hydrophobic peptide fraction, hydrophobic pyroglutamyl peptide fraction, hydrophilic peptide fraction, or hydrophilic pyroglutamyl peptide fraction before forced walking, applied with a load of 3-hr forced walking, then acclimated to an observation cage for 15 minutes, and subsequently i.p. administered with saline, the hydrophobic peptide fraction, hydrophobic pyroglutamyl peptide fraction, hydrophilic peptide fraction, or hydrophilic pyroglutamyl peptide fraction.

(3) Forced walking load

For the forced walking load, the mice were placed in a cage having a diameter of 37 × a depth of 35.5 cm equipped with a motorized running wheel and 3-hr forced walking was conducted at a rate of 1 revolution/25 sec.

(4) Measurement of locomotor activity

Locomotor activity was evaluated by placing each mouse in a plastic cage (length 24 cm × width 17 cm × height 12 cm) and then acclimating to the environment for 15 minutes, followed by automatic conversion of 90-min plane momentum into numerical value using Supermex.

[0030]

[Result]

The results are shown in Figure 1 to Figure 4. Figure 1 to Figure 4 revealed that the hydrophobic

peptide fraction and hydrophobic pyroglutamyl peptide fraction showed better antifatigue effects than the hydrophilic peptide fraction.

[0031]

Example 5

(1) Animal used

For experiments, ddY male mice having body weights of 28 to 32 g (26 g upon delivery) (Japan SLC, Inc.) were used and kept under the constant environment with a room temperature of 22 ± 2°C, a humidity of 55 ± 5% and a 12/12 hour-light/dark cycle (light period; 7:00 to 19:00, dark period 19:00 to 7:00) until subjected to the experiment. A plastic cage (length 30 cm × width 20 cm × height 13 cm) was used while keeping the mice and the mice were fed with pellets and tap water ad libitum when not subjected to the experiment.

(2) Drugs used and method of preparation

· The dipeptide was dissolved in saline and was intraperitoneally (i.p.) administered in an amount of 0.1 mL per 10 g of a body weight. For administration scheme, the mice were i.p. administered with saline or the peptide before forced walking, applied with a load of 3-hr forced walking, then acclimated to an observation cage for 15 minutes, and subsequently i.p. administered with saline or the peptide.

(3) Forced walking load

For the forced walking resistance, the mice were placed in a cage having a diameter of 37 × a depth of

35.5 cm equipped with a motorized running wheel and 3-hr forced walking was conducted at a rate of 1 revolution/25 sec.

(4) Measurement of locomotor activity

Locomotor activity was evaluated by placing each mouse in a plastic cage (length 24 cm × width 17 cm × height 12 cm) and then acclimating to the environment for 15 minutes, followed by automatic conversion of 90-min plane momentum into a numerical value using Supermex.

(5) Statistical processing

The results of experiments were shown by the mean value and standard error (S.E.M). For the significance test, Fisher's PLSD method was carried out after variance analysis processing. A P value of 5% or less was determined that there was significant difference. For this test, Stat view-J 5.0 for Windows (registered trademark) was used.

[0032]

[Results]

The results are shown in Figure 5 to Figure 10. As evident in Figure 5 to Figure 10, Asp-Leu (Dα amino acids), Asp-Leu (Dβ amino acids), Asp-Val (Dα amino acids), Asp-Val (Dβ amino acids), and (D)Asp-Phe (Dα, Dβ) showed good antifatigue effects.

Claims

[Claim 1]

A dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 2]

The dipeptide according to claim 1, wherein the dipeptide is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 3]

A pharmaceutical composition comprising the dipeptide according to claim 1 or 2.

[Claim 4]

The pharmaceutical composition according to claim 3, which is an agent for preventing or improving fatigue.

[Claim 5]

A food product composition comprising the dipeptide according to claim 1 or 2.

[Claim 6]

The food product composition according to claim 5, which is a food product composition for preventing or improving fatigue.

[Claim 7]

Use of the dipeptide according to claim 1 or 2 for producing an agent for preventing or improving fatigue or a food product for preventing or improving fatigue.

[Claim 8]

The dipeptide according to claim 1 or 2 for use in prevention or improvement of fatigue.

[Claim 9]

A method for preventing or improving fatigue, comprising administering an effective amount of the dipeptide according to claim 1 or 2.