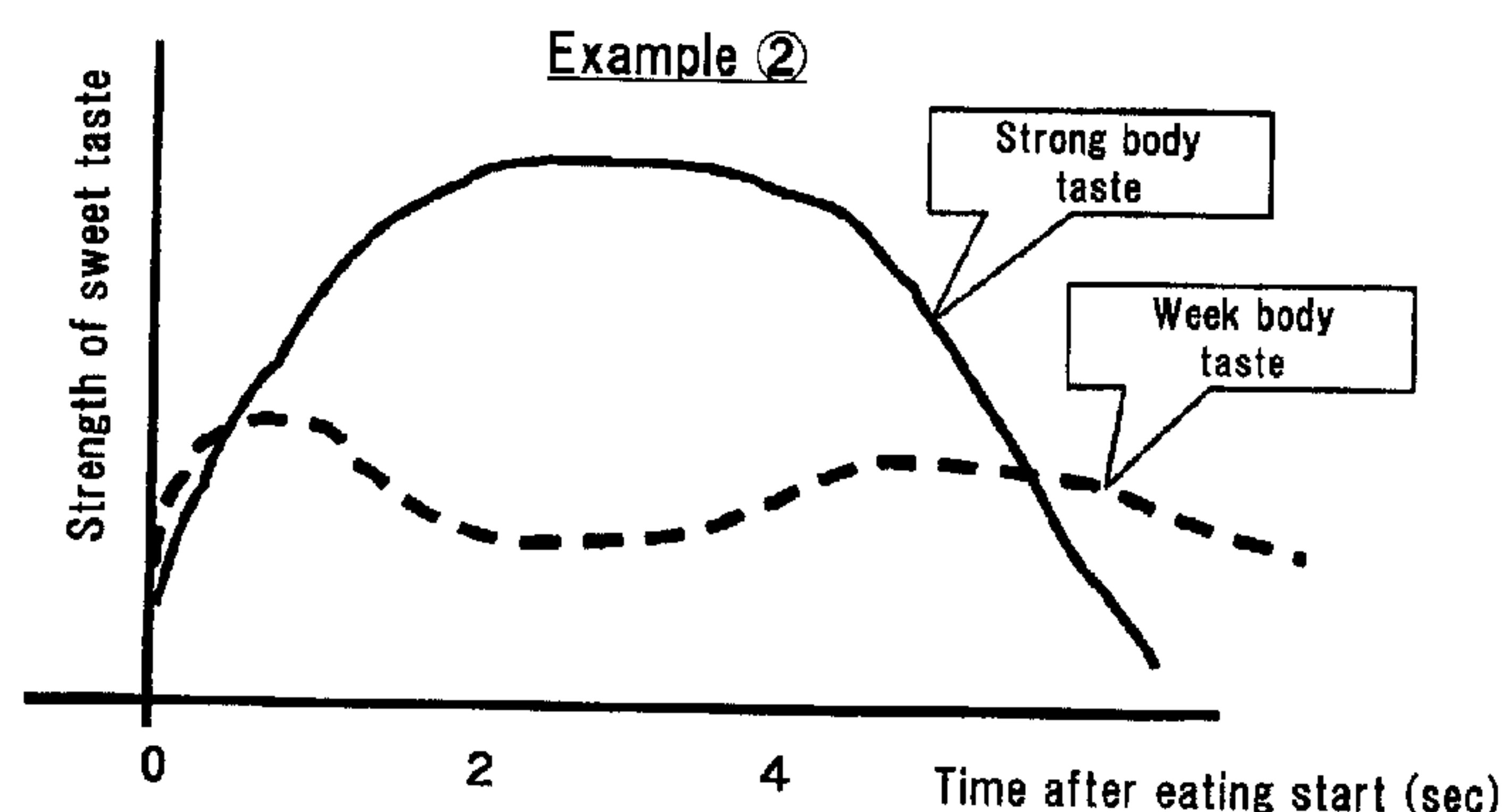
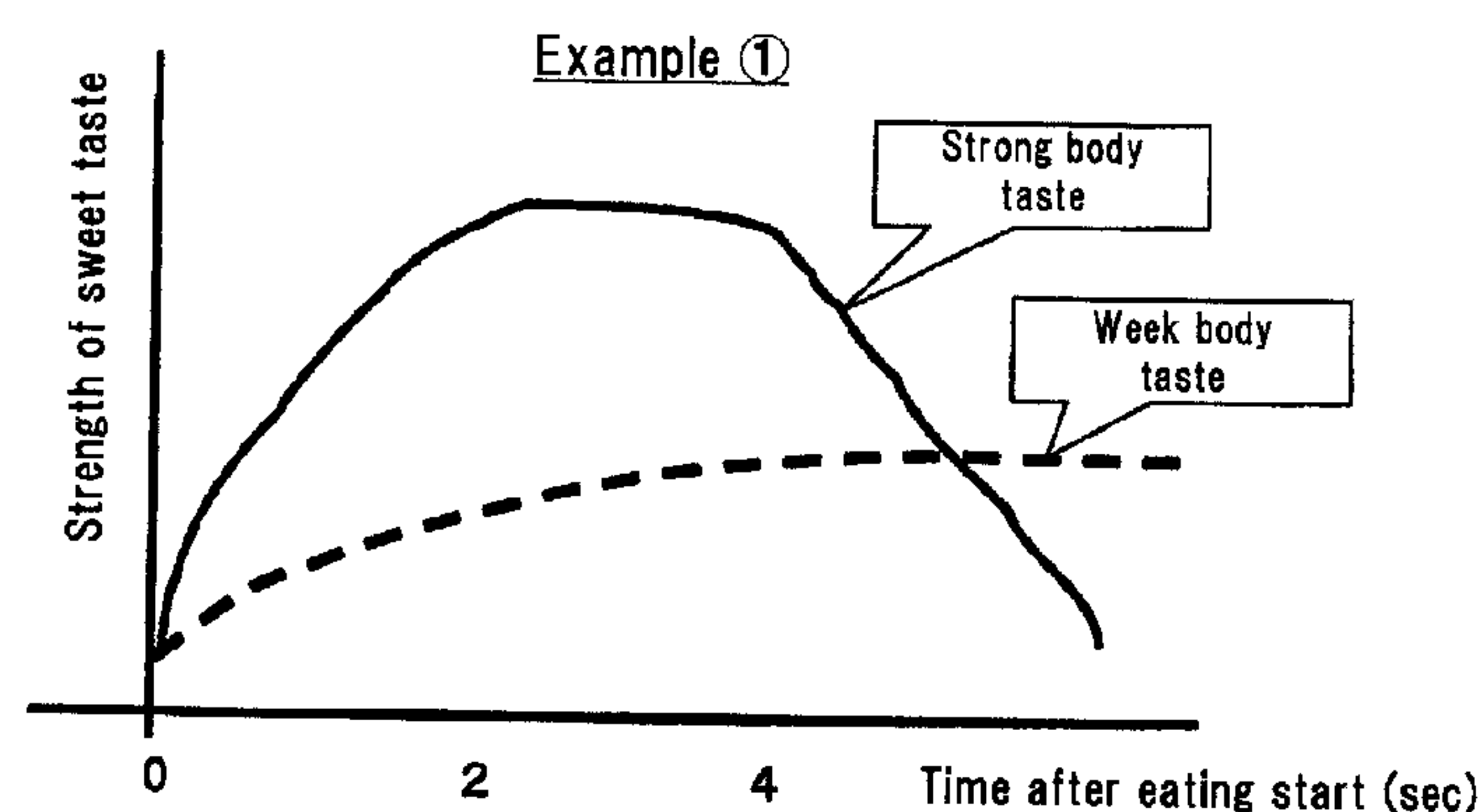




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(54) **Titre : EDULCORANT COMPORTANT UN RECEPTEUR DU CALCIUM**
(54) **Title: A SWEETENER COMPRISING A CALCIUM RECEPTOR**



(57) **Abrégé/Abstract:**

The body taste of a sweetener (for example, aspartame, sucralose, acesulfame, etc.) is improved by blending a sweet substance with an amino acid or a peptide having an effect of activating a calcium receptor such as γ -Glu-X-Gly (wherein X represents an amino acid or an amino acid derivative) , γ -Glu-Val-Y (wherein Y represents an amino acid or an amino acid derivative) , γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met (O) , γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys (S-Me) (O) , γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu, γ -Glu-Cys (S-Me) , etc.

ABSTRACT

The body taste of a sweetener (for example, aspartame, sucralose, acesulfame, etc.) is improved by blending a sweet substance with an amino acid or a peptide having an effect of activating a calcium receptor such as γ -Glu-X-Gly (wherein X represents an amino acid or an amino acid derivative), γ -Glu-Val-Y (wherein Y represents an amino acid or an amino acid derivative), γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu, γ -Glu-Cys(S-Me), etc.

DESCRIPTION

A SWEETENER COMPRISING A CALCIUM RECEPTOR

Technical Field

[0001]

The present invention relates to a sweetener and an additive agent for improving the body taste of a sweet substance used as an ingredient of the sweetener, and improving the bitter taste typical of the sweet substance.

Background Art

[0002]

In recent years, a high intensity sweetener has been utilized as a sweetener in a wide range of food field such as diet foods because of its high sweetness magnification. In the fields of beverages and confectioneries each consuming an especially large amount of sweeteners, various high intensity sweeteners have been used as so-called lower-calorie sweeteners such as a calorie-reduced sweetener and a calorie-free sweetener, and sugar-free sweeteners. Of those, typical examples of the high intensity sweetener to be used include aspartame (APM), sucralose, acesulfame-K (Ace-K), neotame, N- [N- [3- (3-hydroxy-4-methoxyphenyl)propyl]- α -aspartyl]-L-phenyl alanine 1-methyl ester (hereinafter abbreviated as "ANS9801"), saccharin, stevia, glycyrrhizin, thaumatin and monellin.

[0003]

However, in general, consumers have become familiar over the years with the sweet taste of sugar, glucose fructose syrup or the like, which has been used for foods in the past, and hence do not prefer in many cases the quality of sweet taste of such a high intensity sweetener as described above. For example, APM has a utilization effect such as a flavor-enhancing effect and an effect for reducing the bitter taste or the astringency of

coexisting substances, while has a problem in its strong after sweet taste (strong sweet taste in aftertaste) and storage stability such as heat resistance. Sucralose has a high stability, while has a problem in its flavor suppression effect and heavy sweet taste in aftertaste, for example. Ace-K has a high stability, while has a heavy taste in middle taste to after sweet taste (heavy sweet taste over aftertaste) and particularly has a strong bitter taste and astringency, therefore, Ace-K is often used in a combination with other high intensity sweeteners.

[0004]

Until now, the use of those high intensity sweeteners in combination is known as a technology for modifying the quality of sweetness of the above-mentioned high intensity sweetener (Patent Document 1). Further, the use of those high intensity sweeteners in a combination with other sweeteners such as trehalose and erythritol (Patent Document 2) or an α -glucosylated stevia extract (Patent Document 3) and the use in a combination with a dietary fiber (Patent Document 4) have been reported. However, at the present time, these have not yet sufficiently satisfied preferences of consumers who have become familiar with the quality of sweetness of sugar or glucose fructose syrup.

[0005]

Meanwhile, the calcium receptor which is also called Calcium Sensing Receptor (CaSR) is a receptor consisting of 1,078 amino acids, which is classified into the class C of seven-transmembrane receptors (G protein-coupled receptor; GPCR). Cloning of the gene for the calcium receptor was reported in 1993 (Non-patent document 1), and the calcium receptor is known to cause various cell responses via elevation of intracellular calcium level etc., when being activated with calcium etc. The nucleotide sequence of the human calcium receptor is registered with GenBank Accession No. NM_000388 and is well conserved in animals.

[0006]

The above-mentioned calcium receptor may act to promote or suppress biological functions. Therefore, at present, a therapeutic agent that utilizes an activating effect on the calcium receptor and a therapeutic agent that utilizes an inhibitory effect on the calcium receptor are appropriately used in the treatment of neurological diseases, hepatic diseases, cardiovascular diseases, digestive system diseases and other diseases, depending on pathological conditions. For example, the calcium receptor has functions to detect increased blood calcium level in the parathyroid, and suppress the secretion of the parathyroid hormone (PTH) to correct the blood calcium level. Therefore, an effect of reducing the blood calcium level is expected for a calcium receptor activator. It has been actually clarified that when the calcium receptor activator is used to treat secondary hyperparathyroidism in a hemodialysis patient, it reduces the PTH level without increasing the calcium and phosphorus levels.

[0007]

Since the functional analysis of the calcium receptor has been conducted mainly for calcium homeostasis, the applied researches therefore, have so far mainly focused on bone metabolic diseases in which calcium regulation is involved. However, it has become clear that the calcium receptor is widely distributed in living bodies other than the parathyroid and kidney from the results of genetic expression analyses etc. (Non-patent documents 2 and 3), and possibilities thereof for being involved in various biological functions and causes of diseases have been proposed. For example, it is speculated that the calcium receptor is involved in the functions of the liver, heart, lung, gastrointestinal tract, lymphocyte and pancreas. The inventors of the present invention also have confirmed that the calcium receptor is expressed in a wide range of tissues in living bodies by analyses by analyzing RNAs extracted from each of rat tissues using RT-PCR. From the above-mentioned viewpoints, the values of activators and inhibitors of the calcium receptor are presently rapidly increasing for applications.

[0008]

Moreover, in addition to calcium, cations such as a gadolinium cation, basic peptides such as polyarginine, polyamine such as spermine, amino acids such as phenylalanine, and so forth have been reported as calcium receptor activators (Non-patent document 4).

Non-patent Document 5 reports that glutathione (γ -Glu-Cys-Gly), a low molecular weight peptide, is a CaSR activator, but does not mention in any way a possibility for CaSR to be involved in taste reception.

[0009]

However, it is not known that an amino acid or a peptide having a particular structure is useful as a calcium receptor activator. Further, although a peptide derivative which exhibits a sweet taste such as aspartame is known, it is not known that an amino acid or peptide having a calcium receptor-activating effect may improve a sweet taste of a sweet substance. It should be noted that ANS9801 and neotame are described in Patent Documents 5 and 6, respectively.

[Patent Document 1] JP 2005-304440 A

[Patent Document 2] JP 2002-51723 A

[Patent Document 3] JP 2002-34501 A

[Patent Document 4] JP 2004-41118 A

[Patent Document 5] US 6,548,096

[Patent Document 6] International Publication NO. WO39979 pamphlet

[Non-patent Document 1] Nature, 1993, Vol. 366(6455), pp. 575-580

[Non-patent Document 2] J. Endocrinol., 2000, Vol. 165(2), pp. 173-177

[Non-patent Document 3] Eur. J. Pharmacol., 2002, Vol. 447(2-3), pp. 271-278

[Non-patent Document 4] Cell Calcium, 2004, Vol. 35(3), pp. 209-216

[Non-patent Document 5] J. Biol. Chem., 2006, Vol. 281(13), pp. 8864-8870

Disclosure of the Invention

Problems to be solved by the Invention

[0010]

Objects of the present invention are: 1) to provide a sweetener in which the sweet taste quality, in particular, the body taste and bitter taste has improved, especially, a high intensity sweetener in which the sweet taste quality, in particular, the body taste and bitter taste has improved; and 2) to provide an additive agent for improving the body taste of a sweet substance which is used as an ingredient of the sweetener, and improving the bitter taste typical of the sweet substance.

Means for solving the Problems

[0011]

The inventors of the present invention found various amino acids and peptides as compounds each having a calcium receptor-activating effect in the process of searching a calcium receptor activator, and also found that the amino acids and peptides can improve the quality of sweetness of a high intensity sweetener, thereby accomplished the present invention.

That is, the present invention is as follows:

- (1) a sweetener comprising a sweet substance and a compound having calcium receptor-activating effect;
- (2) the sweetener according to (1) wherein the compound comprises one or more kinds of amino acid or peptide selected from the group consisting of γ -Glu-X-Gly where X represents an amino acid or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me);
- (3) the sweetener according to (2) wherein X represents Cys(SNO), Cys(S-allyl), Gly, Cys(S-Me), Abu, t-Leu, Cle, Alb, Pen or Ser, and Y represents Gly, Val, Glu, Lys, Phe, Ser, Pro, Arg, Asp, Met,

Thr, His, Orn, Asn, Cys, Gln, GlyA or LacA;

(4) the sweetener according to (2) or (3) wherein the amino acid or peptide is selected from γ -Glu-Val-Gly and γ -Glu-Abu-Gly;

(5) the sweetener according to any one of (1) to (4) wherein the sweet substance comprises one or more kinds of substance selected from the group consisting of aspartame, sucralose, acesulfame K, neotame, ANS9801, saccharin, stevia, glycyrrhizin, thaumatin, monatin and monellin;

(6) an additive agent comprising a compound having calcium receptor-activating effect, which is added to a sweet substance for improving a body taste of the sweet substance; and

(7) an additive agent comprising a compound having calcium receptor-activating effect, which is added to a sweet substance for improving a bitter taste of the sweet substance.

Brief Description of the Drawings

[0012]

FIG. 1 is a graph showing an effect of calcium on a calcium receptor. cRNA of the human calcium receptor was injected into oocyte of *Xenopus laevis* by microinjection. Values of intracellular response currents which flew at the time of addition of a calcium chloride solution at an arbitrary concentration were recorded. The maximum values of intracellular currents were considered response current values. It was confirmed that no response was observed in oocytes injected with distilled water by microinjection as a control.

FIG. 2 is a graph showing an effect of an L-amino acid on a calcium receptor. cRNA of the human calcium receptor was injected into oocyte of *Xenopus laevis* by microinjection. Values of intracellular response currents which flew at the time of addition of a 10 mM L-amino acid solution were recorded. The maximum values of intracellular currents were considered response current values. It was confirmed that no response was observed in oocytes injected with distilled water by microinjection as a control.

FIG. 3 is a graph showing an effect of a D-amino acid on a

calcium receptor. cRNA of the human calcium receptor was injected into oocyte of *Xenopus laevis* by microinjection. Values of intracellular response currents which flew at the time of addition of a 10 mM D-amino acid solution were recorded. The maximum values of intracellular currents were considered response current values. It was confirmed that no response was observed in oocytes injected with distilled water by microinjection as a control.

FIG. 4 is a graph showing an effect of a peptide on a calcium receptor. cRNA of the human calcium receptor was injected into oocyte of *Xenopus laevis* by microinjection. Values of intracellular response currents which flew at the time of addition of a peptide solution at an arbitrary concentration. The maximum values of intracellular currents were considered response current values. It was confirmed that no response was observed in oocytes injected with distilled water by microinjection as a control.

FIG. 5 is a conceptual diagram showing strength of a body taste of a sweet substance.

Best Mode for carrying out the Invention

[0013]

Hereinafter, the present invention is described in detail.

One embodiment of the present invention relates to a sweetener containing a sweet substance and a compound, preferably an amino acid or a peptide, having calcium receptor-activating effect. Further, another embodiment of the present invention relates to an additive agent containing a compound having calcium receptor-activating effect, which is added to a sweet substance in order to improve the body taste and bitter taste of the sweet substance. Further, still another embodiment of the present invention relates to a sweetener which is obtained by adding the additive agent containing a compound having calcium receptor-activating effect.

[0014]

First, the amino acid or the peptide having calcium

receptor-activating effect is described.

The inventors of the present invention have found that the amino acid or the peptide having calcium receptor-activating effect can impart kokumi to a food. Further, the inventors of the present invention have found that the amino acid or the peptide can improve the body taste and the bitterness of the sweet substance. It should be noted that the term "kokumi" means a taste that cannot be expressed by five basic tastes, which are sweet taste, salty taste, sour taste, bitter taste and umami, and means a taste in which marginal tastes of the basic tastes, such as thickness, growth (mouthfulness), continuity and harmony as well as the basic tastes are enhanced. [0015]

Meanwhile, the term "body taste" of the sweet substance as used herein means that the sweet substance has harmony and richness mainly in initial taste and middle taste when eating the sweet substance. The phrase "improvement of the body taste" refers to the enhancement of the body taste. The sense of taste varies with the course of time after the start of eating, and is sequentially called immediately after the start of eating, initial taste, middle taste and aftertaste. Those are relative notions, and typically, initial taste, middle taste and aftertaste refer to tastes which are felt 0 to 2 seconds, 3 to 4 seconds, and 5 seconds or later after the start of eating, respectively. FIG. 5 shows a conceptual diagram showing the body taste, in which the ordinate axis represents the strength of the taste, and the abscissa axis represents the time after the start of eating. However, FIG. 5 is a conceptual diagram and does not show absolute values.

[0016]

The term "calcium receptor" as used herein refers to a receptor that is called Calcium Sensing Receptor (CaSR) and belongs to the class C of seven-transmembrane receptors. The term "calcium receptor activator" refers to a substance that binds to the above-mentioned calcium receptor to activate the calcium receptor.

Further, the phrase "to activate a calcium receptor" as used herein means that a ligand binds to a calcium receptor to activate a guanine nucleotide binding protein, thereby transmit a signal. Further, the term "calcium receptor activity" means that the calcium receptor transmits the signal.

Herein, each amino acid and amino acids forming each peptide are L-amino acids unless otherwise stated.

[0017]

<1> Compound having a calcium receptor-activating effect

The compound having a calcium receptor-activating effect can be an amino acid, a peptide or derivatives thereof, or various low molecular weight compounds as long as the compound has an effect of improving the sweet taste quality of the sweet substance. The compound can also be a novel compound obtained by screening. For example, the compound can be obtained by reacting a calcium receptor with a test substance, and detecting a calcium receptor activity. It is preferable to confirm that the obtained compound has an effect of improving the quality of sweetness of the sweet substance, in particular, the high intensity sweetener.

[0018]

Hereinafter, a method of screening the compound having a calcium receptor-activating effect is specifically described, but is not limited to the following steps:

- 1) measuring a calcium receptor activity by adding a test substance to a calcium receptor activity measurement system for measuring the calcium receptor activity;
- 2) comparing the calcium receptor activity when adding the test substance with a calcium receptor activity when not adding the test substance; and
- 3) selecting the test substance exhibiting a calcium receptor-activating effect at the time of addition of the test substance.

[0019]

The calcium receptor activity is measured by a measurement system using cells that express calcium receptors, for example. The above-mentioned cells can be cells endogenously expressing calcium receptors, or can be recombinant cells in which an exogenous calcium receptor gene is introduced. The calcium receptor activity measurement system described above may be used without any particular limitation as long as, when an extracellular ligand (activator) specific to a calcium receptor is added to the above-mentioned cells that express calcium receptors, the measurement system may detect the binding (reaction) between the activator and the calcium receptor, or may respond to the binding (reaction) between the activator and the calcium receptor to thereby transmit a detectable signal into the cells. When the calcium receptor activity is detected through the reaction with the test substance, it is clarified that the test substance has a calcium receptor-stimulating activity, and is a substance that may improve the sweet taste quality of the sweet substance.

[0020]

Meanwhile, an effect of improving the quality of sweetness of the sweet substance can be confirmed by a method such as a taste test by humans. Further, the amino acid and peptide to be used as a test substance are not particularly limited; however, the peptide is preferably a peptide consisting of 2 to 10 amino acid residues, or a derivative thereof, and more preferably a peptide consisting of 2 or 3 amino acid residues, or a derivative thereof. Further, the amino acid residue at the N-terminal of the peptide is preferably γ -glutamic acid.

[0021]

Examples of the calcium receptor mentioned above include a calcium receptor derived from an animal such as a mouse, rat and dog as well as human, and the origin of the calcium receptor is not particularly limited.

[0022]

As described above, the calcium receptor activity can be

confirmed by using live cells expressing a calcium receptor or its fragment, cell membranes expressing a calcium receptor or its fragment, an in vitro system containing a calcium receptor or its fragment protein, or the like.

An example using live cells is described below. However, confirmation of the calcium receptor activity is not limited to this example.

[0023]

A calcium receptor is expressed in cultured cells such as those of *Xenopus laevis* oocytes, hamster ovarian cells and human fetal kidney cells. The calcium receptor can be expressed by cloning a calcium receptor gene in a plasmid that carries a foreign gene and introducing the plasmid or cRNA obtained by using the plasmid as a template to the cells. To detect the reaction, an electrophysiological technique and a fluorescent indicator that indicates an increase in intracellular calcium level can be used.

[0024]

Expression of the calcium receptor is first confirmed based on the response to calcium or a specific activator. Oocytes in which intracellular current is observed in response to 5 mM of calcium, or cultured cells in which fluorescence of the fluorescent indicator reagent is observed in response to 5 mM of calcium are used. The calcium concentration dependency is determined by changing the calcium concentration. Then, a test substance such as a peptide is prepared to a concentration of about 1 μ M to 1 mM, and added to the oocytes or cultured cells, and the calcium receptor activity of the above-mentioned peptide or the like is determined.

[0025]

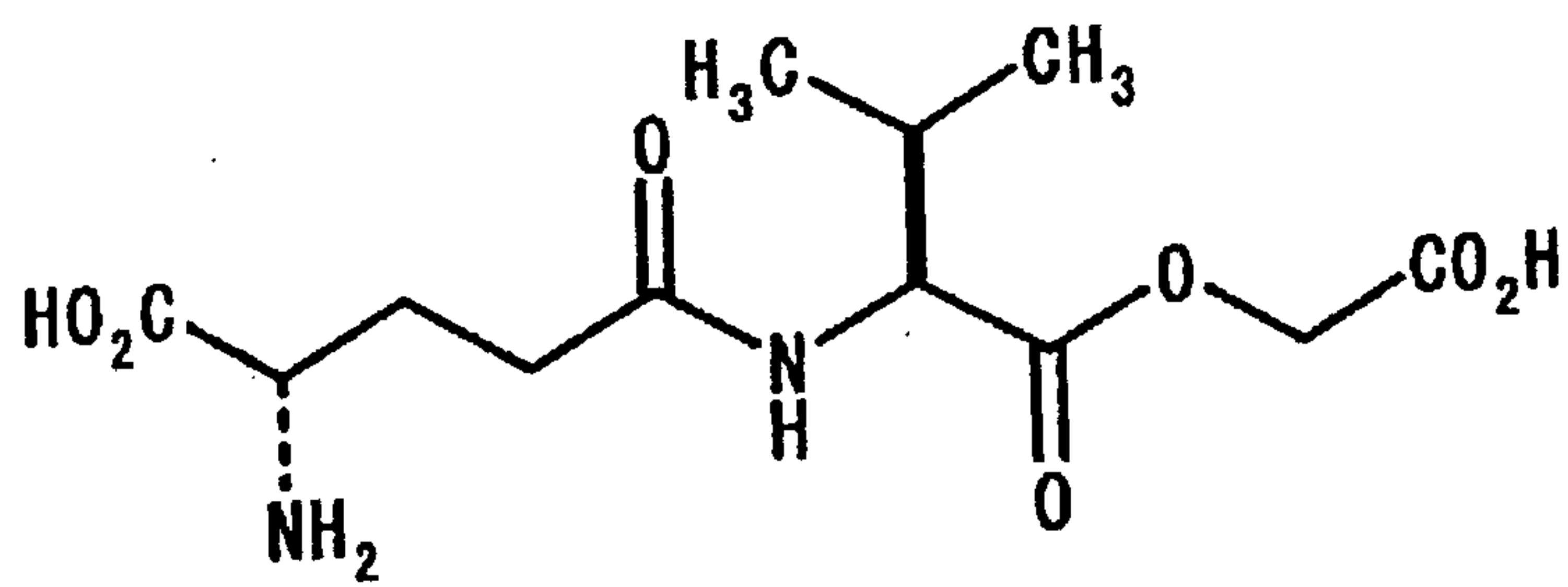
Examples of the compound to be used in the present invention (hereinafter, referred to as a "compound of the present invention") include various amino acids, peptides or derivatives thereof, or various low molecular weight compounds, each of which has a calcium receptor-activating effect (hereinafter, when "amino acid" or "peptide" is simply used, the amino acid and the peptide sometimes

mean both of an amino acid and an amino acid derivative, and both of a peptide and a peptide derivative, respectively). The amino acid or the peptide having a calcium receptor-activating effect to be used in the present invention can be an amino acid or a peptide having an effect of improving the body taste and/or bitterness of the sweet substance, when the amino acid or the peptide is eaten with the sweet substance. Examples of such an amino acid or a peptide include γ -Glu-X-Gly where X represents an amino acid or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me) (hereinafter, also referred to as a "peptide or the like of the present invention"). In the present invention, one kind of those peptides or the like can be used, or two or more kinds thereof can be used in combination.

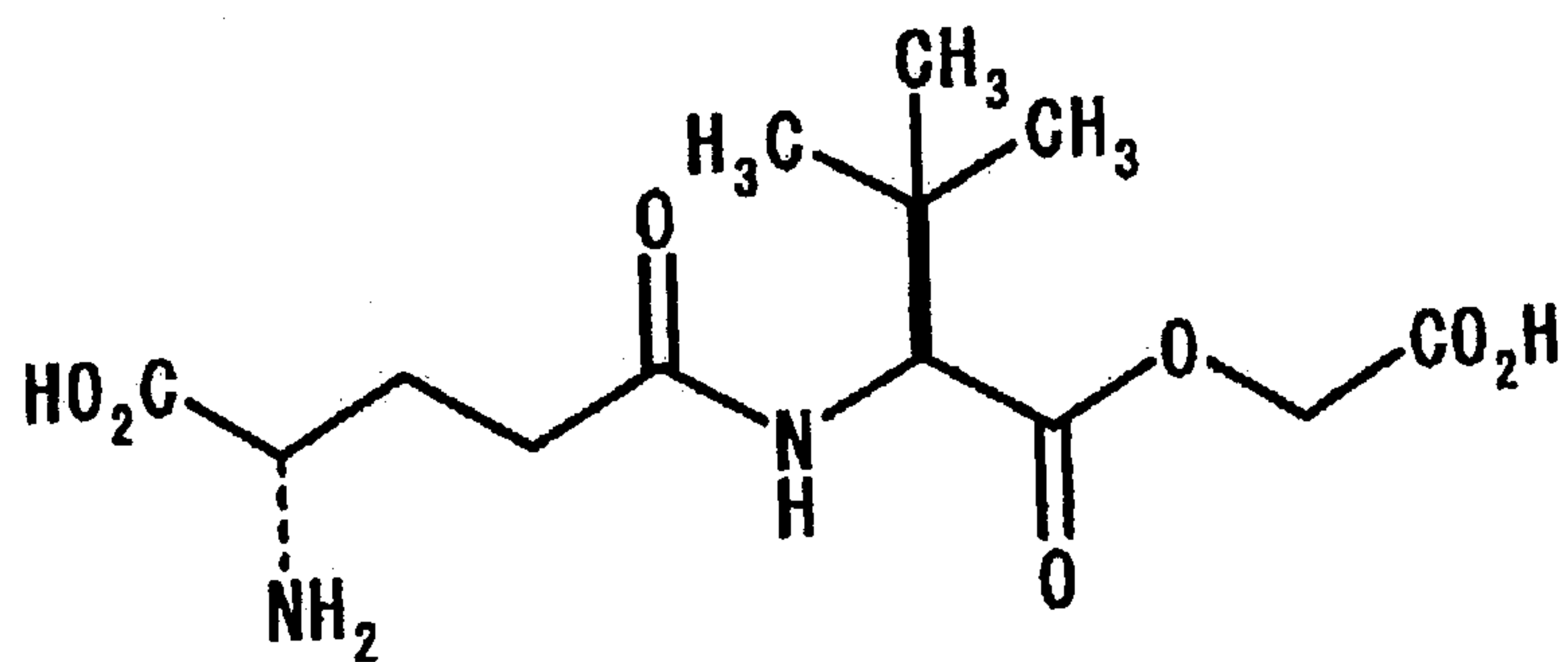
[0026]

Further, the peptide may be a peptide derivative having a structure of γ -Glu-X-OCH(Z)CO₂H (X represents an amino acid or an amino acid derivative, and Z represents H (a hydrogen atom) or CH₃ (a methyl group)). Further, the peptide may be a compound in which Y represents GlyA or LacA in the formula γ -Glu-Val-Y. Preferable specific examples thereof include γ -Glu-Val-GlyA, γ -Glu-tLeu-GlyA, γ -Glu-Abu-GlyA, γ -Glu-Val-LacA, γ -Glu-tLeu-LacA and γ -Glu-Abu-LacA. It should be noted that GlyA represents glycolic acid, and LacA represents lactic acid. Lactic acid may be one of S-lactic acid or R-lactic acid, and preferred is S-lactic acid. Structural formulae of those compounds are described below.

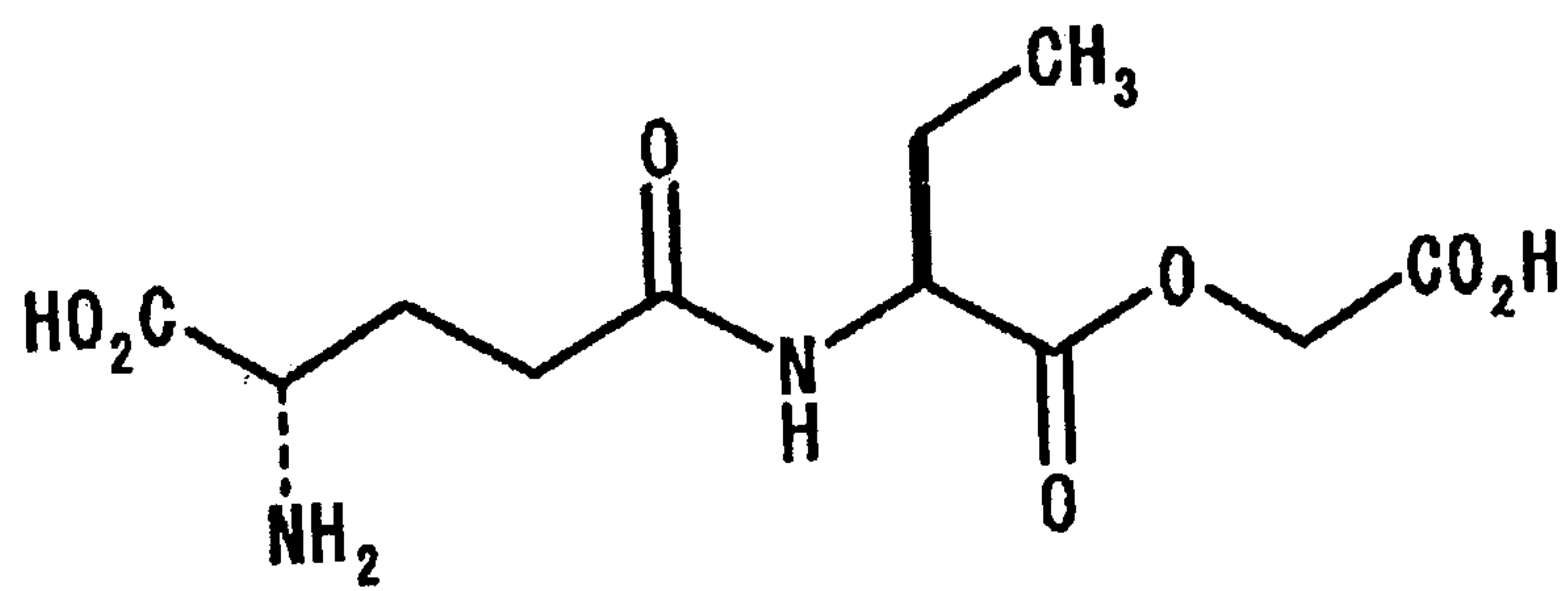
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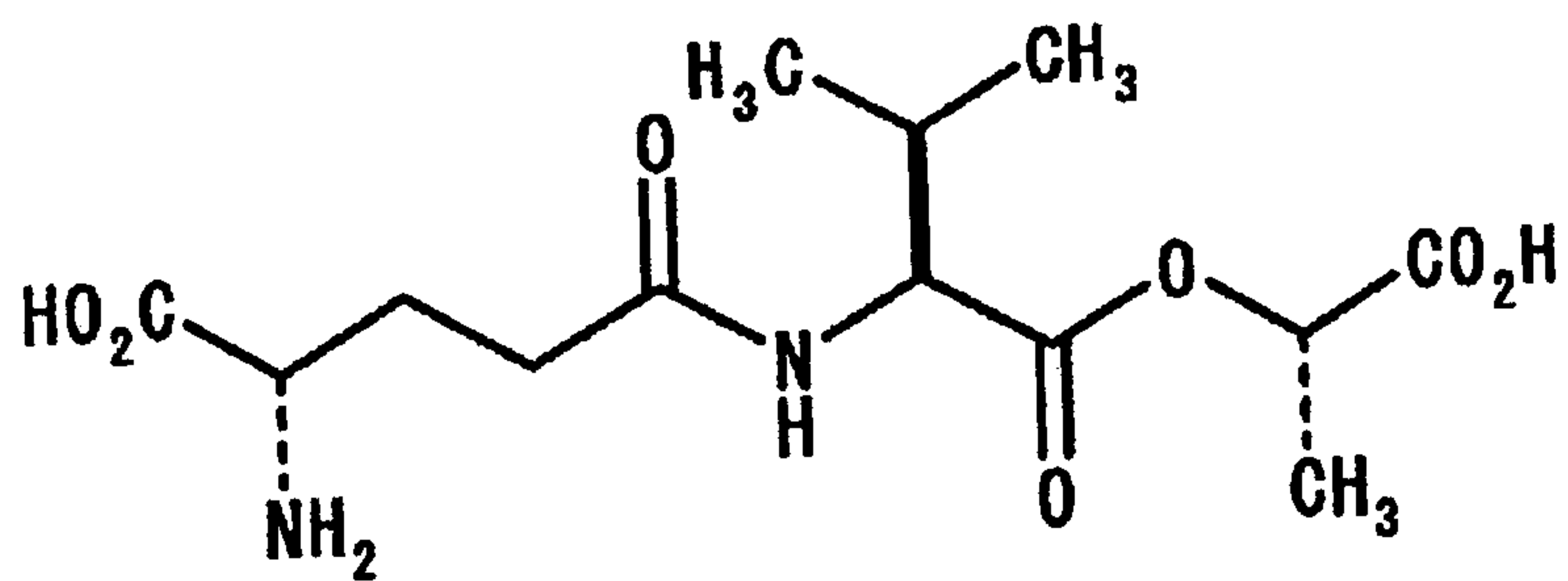
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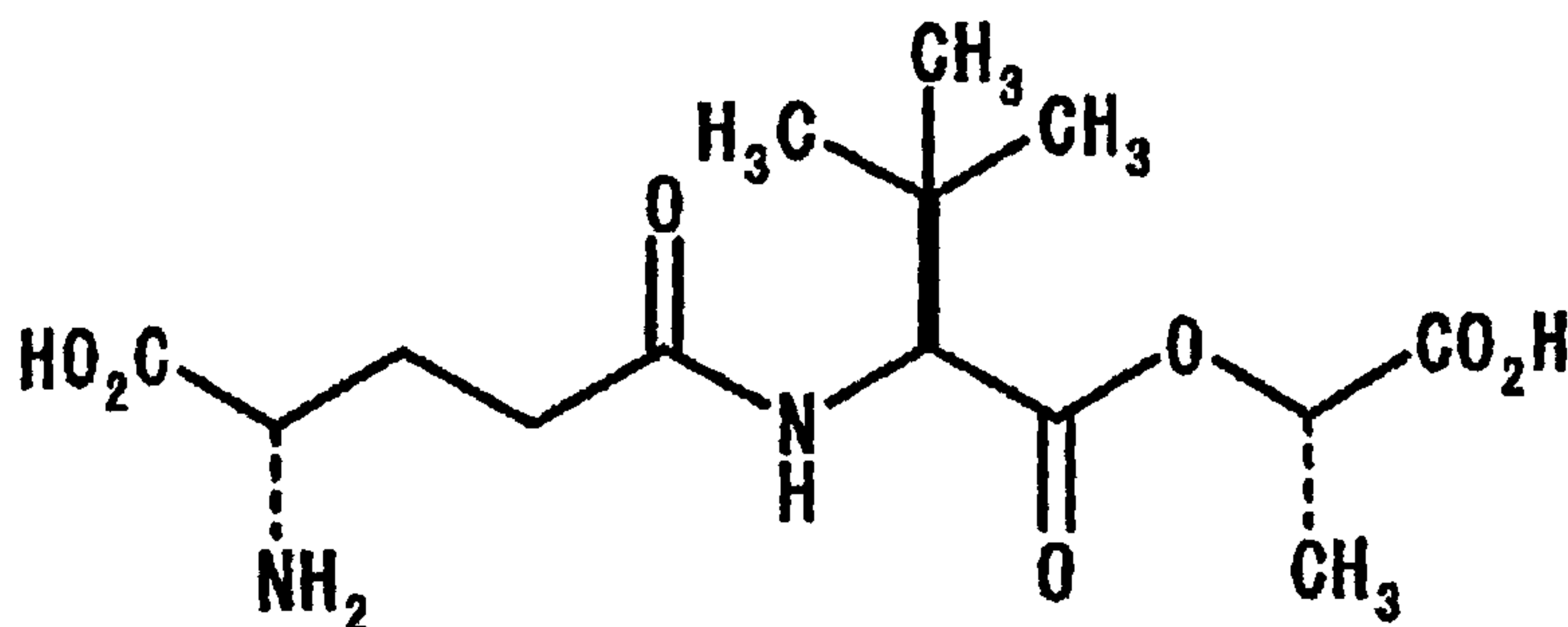
[0029]



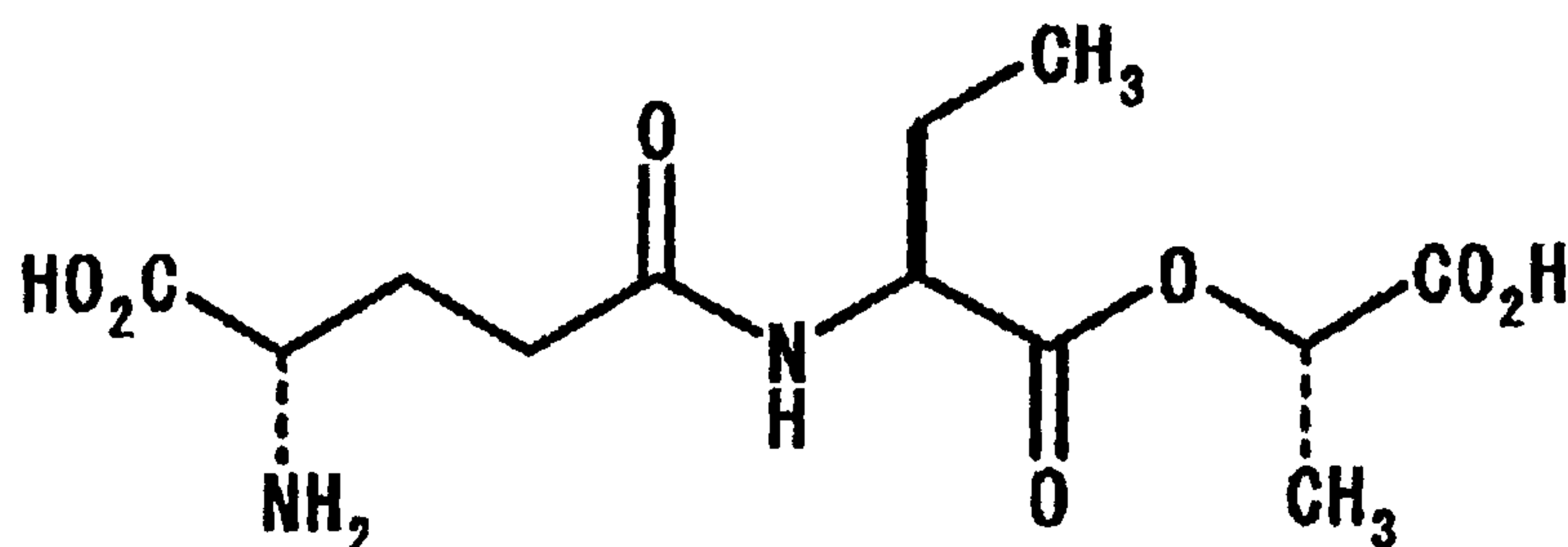
[0030]



[0031]



[0032]



[0033]

Herein, examples of the amino acid include a neutral amino acid such as Gly, Ala, Val, Leu, Ile, Ser, Thr, Cys, Met, Asn, Gln, Pro, Hyp and t-Leu, an acidic amino acid such as Asp and Glu, a basic amino acid such as Lys, Arg and His, an aromatic amino acid such as Phe, Tyr and Trp, and homoserine, citrulline, ornithine, α -aminobutyric acid, norvaline, norleucine and taurine. The amino acid can also be a non-naturally occurring (non-protein constituent) amino acid such as *tert*-leucine, cycloleucine, α -aminoisobutyric acid and L-penicillamine. It should be noted that X in the peptide γ -Glu-X-Gly can be any one of above-mentioned amino acid or a derivative thereof, and preferably an amino acid or a derivative thereof, other than Cys.

[0034]

Herein, abbreviations for amino acid residues mean the following amino acids.

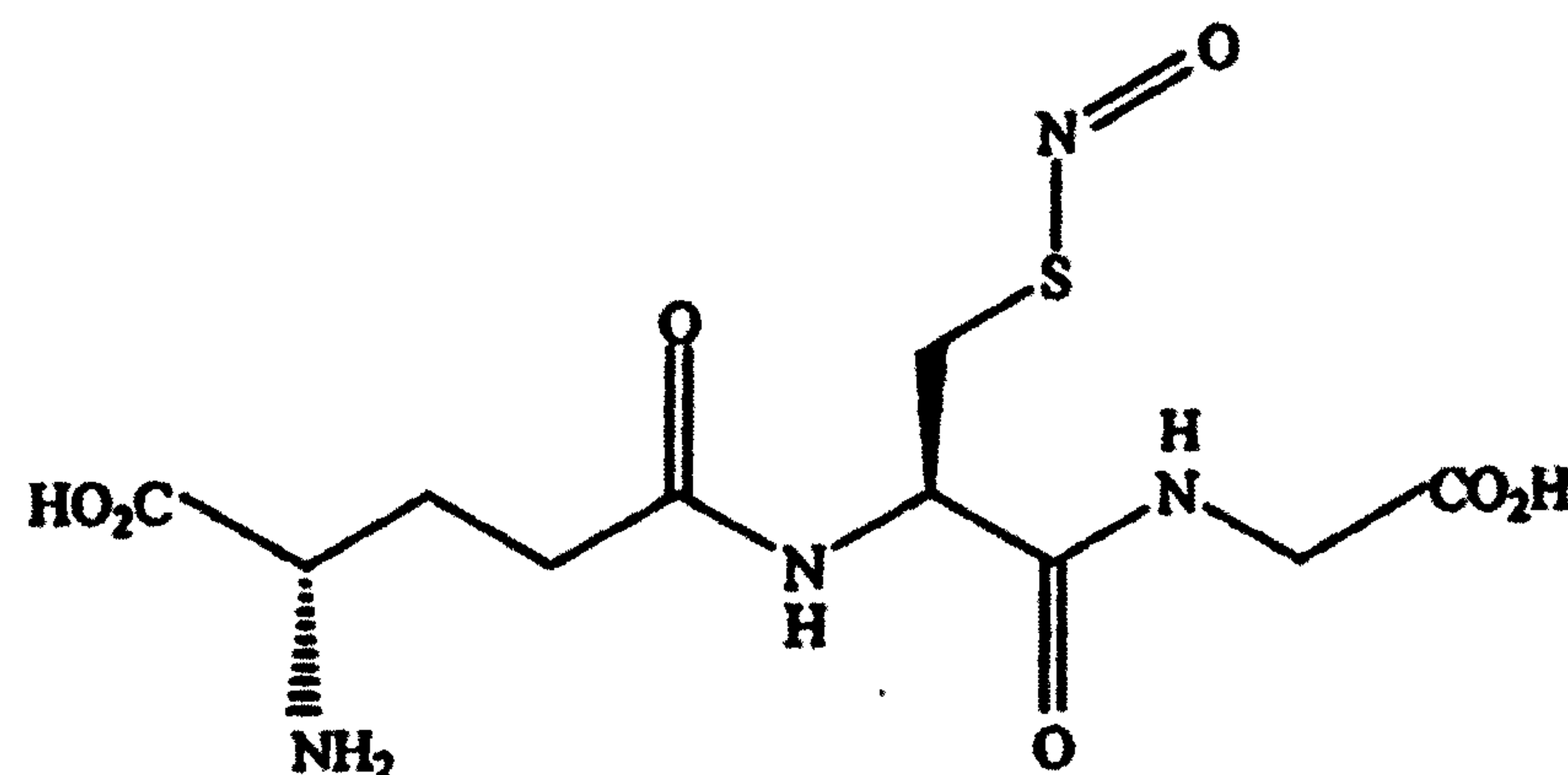
- (1) Gly: Glycine
- (2) Ala: Alanine

- (3) Val: Valine
 - (4) Leu: Leucine
 - (5) Ile: Isoleucine
 - (6) Met: Methionine
 - (7) Phe: Phenylalanine
 - (8) Tyr: Tyrosine
 - (9) Trp: Tryptophan
 - (10) His: Histidine
 - (11) Lys: Lysine
 - (12) Arg: Arginine
 - (13) Ser: Serine
 - (14) Thr: Threonine
 - (15) Asp: Aspartic acid
 - (16) Glu: Glutamic acid
 - (17) Asn: Asparagine
 - (18) Gln: Glutamine
 - (19) Cys: Cysteine
 - (20) Pro: Proline
 - (21) Orn: Ornithine
 - (22) Sar: Sarcosine
 - (23) Cit: Citrulline
 - (24) N-Val: Norvaline
 - (25) N-Leu: Norleucine
 - (26) Abu: α -Aminobutyric acid
 - (27) Tau: Taurine
 - (28) Hyp: Hydroxyproline
 - (29) t-Leu: tert-Leucine
 - (30) Cle: Cycloleucine
 - (31) Aib: α -Aminoisobutyric acid (2-methylalanine)
 - (32) Pen: L-Penicillamine
- [0035]

Further, examples of the amino acid derivative include various derivatives of above-mentioned amino acids such as a special amino acid, a non-natural amino acid, an amino alcohol and a substituted

amino acid of which an amino acid side chain such as the terminal carbonyl group, the terminal amino group, and the thiol group of cysteine, is substituted with various substituents. Examples of the substituent include an alkyl group, an acyl group, a hydroxy group, an amino group, an alkylamino group, a nitro group, a sulfonyl group and various protective groups, specifically include Arg(NO₂): N-γ-nitroarginine, Cys(SNO): S-nitrocysteine, Cys(S-Me): S-methylcysteine, Cys(S-allyl): S-allylcysteine, Val-NH₂: valinamide, Val-ol: valinol (2-amino-3-methyl-1-butanol).
[0036]

It should be noted that herein, γ-Glu-Cys(SNO)-Gly has the following structural formula, and the "(O)" in the above formulae γ-Glu-Met(O) and γ-Glu-Cys(S-Me)(O) indicates a sulfoxide structure. The "(γ)" in the formula γ-Glu indicates that glutamic acid binds to another amino acid via the carboxy group at the γ-position in glutamic acid.



S-Nitrosoglutathione (GNSO)

[0038]

γ-Glu-X-Gly where X represents an amino acid or an amino acid derivative, γ-Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ-Glu-Ala, γ-Glu-Gly, γ-Glu-Cys, γ-Glu-Met, γ-Glu-Thr, γ-Glu-Val, γ-Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ-Glu-Met(O), γ-Glu-γ-Glu-Val, γ-Glu-Val-NH₂, γ-Glu-Val-ol, γ-Glu-Ser, γ-Glu-Tau,

γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me) each improve the body taste and bitterness of the sweet substance.

Therefore, γ -Glu-X-Gly where X represents an amino acid or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me) (hereinafter, also referred to as "peptide and amino acid to be used in the present invention") can be used as an additive agent for a sweet substance which is added to a sweet substance for improving the body taste and bitterness of the sweet substance.
[0039]

The compound to be used in the present invention can be used alone or as a mixture of arbitrary two or more kinds. Of those, a compound having the following structural formula: γ -Glu-X-Gly where X represents Cys(SNO), Cys(S-allyl), Gly, Cys(S-Me), Abu, t-Leu, Cle, Aib, Pen or Ser, or γ -Glu-Val-Y where Y represents Gly, Val, Glu, Lys, Phe, Ser, Pro, Arg, Asp, Met, Thr, His, Orn, Asn, Cys, Gln, GlyA or LacA is preferably used.

Of those compounds, preferred compounds are γ -Glu-Val-Gly and γ -Glu-Abu-Gly.

[0040]

As the above-mentioned compound to be used in the present invention, a compound which is commercially-available can be used. Further, when the compound was a peptide, the peptide can be obtained by appropriately using a known technique such as (1) a method of chemically synthesizing the peptide or (2) a method of synthesizing the peptide by an enzymatic reaction. Since the number of amino acid residues contained in the peptide to be used in the present invention is as comparatively small as 2 or 3 residues, a method

of chemically synthesizing the peptide is convenient. When the peptide is chemically synthesized, the oligopeptide can be synthesized or semi-synthesized by using a peptide synthesizer. An example of the method of chemically synthesizing the peptide includes a peptide solid phase synthetic method. The peptide synthesized as described above can be purified by usual means such as ion exchange chromatography, reversed phase high performance liquid chromatography or affinity chromatography. Such a peptide solid phase synthetic method and the subsequent peptide purification are well known in the technical field.

[0041]

Further, the peptide to be used in the present invention can also be produced by an enzymatic reaction. For example, the method described in International Publication NO.WO2004/011653 pamphlet can be used. That is, the peptide can also be produced by reacting an amino acid or dipeptide in which carboxyl terminus is esterified or amidated with an amino acid having a free amino group (for example, an amino acid in which carboxyl group is protected) in the presence of a peptide producing enzyme, and purifying the produced dipeptide or tripeptide. Examples of the peptide producing enzyme include a culture of a microorganism having an ability to produce a peptide, microbial cells separated from the culture or a processed product of cells of the microorganism, or a peptide producing enzyme derived from the microorganism.

It should be noted that the peptide to be used in the present invention is not only produced by such an enzymatic method or a chemical synthesis method as mentioned above, but also may exist in, for example, a plant such as a vegetable or a fruit, a microorganism such as a yeast and a yeast extract. When the peptide exists in natural products, the peptide which is extracted from those natural products can be used.

Further, the peptide does not need to be isolated before use, and there can be used as a fraction containing the peptide of the present invention in a large amount.

[0042]

Examples of the low molecular weight compound to be used in the present invention include cinacalcet

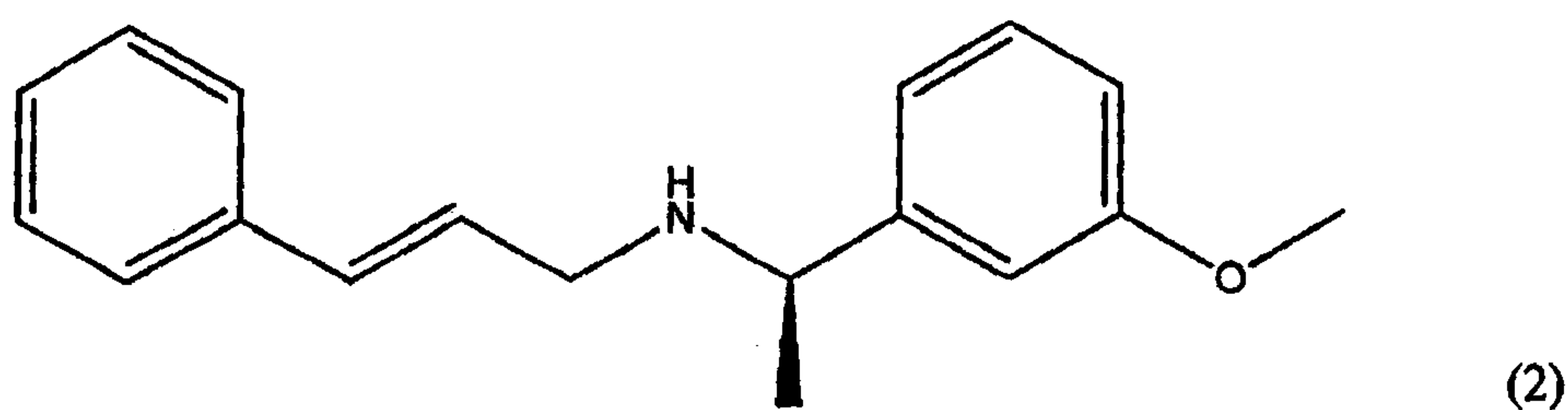
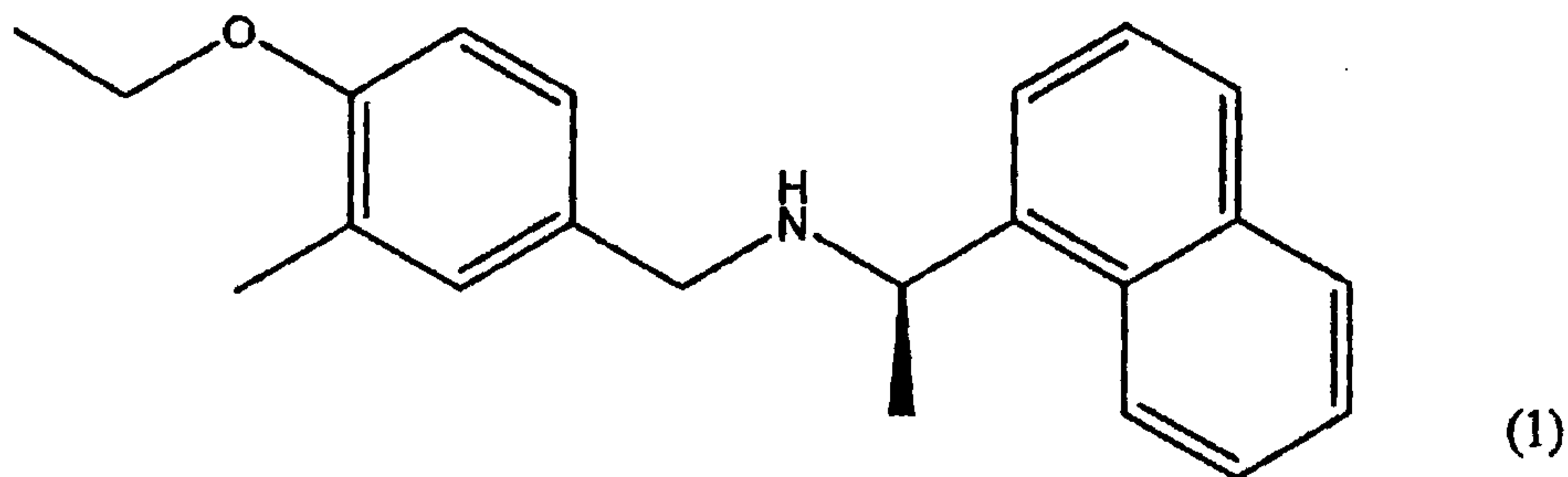
((R)-N-(3-(3-(trifluoromethyl)phenyl)propyl)-1-(1-naphthyl)ethylamine) and an analogous compound thereof. Examples of the analogous compound of cinacalcet include the compound represented by the following chemical formula (1);

((R)-N-[(4-ethoxy-3-methylphenyl)methyl]-1-(1-naphthyl)ethylamine)), or the compound represented by the following chemical formula (2);

((R)-N-(3-phenylprop-2-enyl)-1-(3-methoxyphenyl)ethylamine).

Those compounds can be synthesized by such a known method as described in US Patent No. 6,211,244, for example. Further, commercially-available products can also be used.

[0043]



[0044]

The compound to be used in the present invention also includes that in the form of a salt. When the peptide and the amino acid to be used in the present invention are in the form of a salt, the salt can be a pharmacologically acceptable salt. Examples of a salt with an acidic group such as a carboxyl group in the formula include an ammonium salt, a salt with an alkali metal such as sodium and

potassium, a salt with an alkaline earth metal such as calcium and magnesium, an aluminum salt, a zinc salt, a salt with an organic amine such as triethylamine, ethanolamine, morpholine, pyrrolidine, piperidine, piperazine and dicyclohexylamine, and a salt with a basic amino acid such as arginine and lysine. Examples of a salt with a basic group in case where the basic group exists in the formula include a salt with an inorganic acid such as hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid and hydrobromic acid, a salt with an organic carboxylic acid such as acetic acid, citric acid, benzoic acid, maleic acid, fumaric acid, tartaric acid, succinic acid, tannic acid, butyric acid, hibenzoic acid, pamoic acid, enanthoic acid, decanoic acid, teoclic acid, salicylic acid, lactic acid, oxalic acid, mandelic acid and malic acid, and a salt with an organic sulfonic acid such as methanesulfonic acid, benzenesulfonic acid and p-toluenesulfonic acid.

[0045]

<2> Additive agent and sweetener of present invention

The compound to be used in the present invention, preferably the peptide and the amino acid, can be used as an additive agent which is added to a sweet substance in order to improve the body taste of the sweet substance. The peptide and the amino acid to be used in the present invention can also be used as an additive agent which is added to a sweet substance in order to improve the bitterness of the sweet substance.

The compound can be used alone or as a mixture of two or more kinds. The compound to be used in the present invention can also be mixed with a sweet substance to form a sweetener.

[0046]

The additive agent of the present invention can consist of only one kind or two or more kinds of compounds chosen from the above-mentioned compound to be used in the present invention. Further, the additive agent can be formed by optionally adding other compounds or various additives each having an effect of improving the quality of sweetness of the sweetener.

[0047]

The amount of the additive agent which is added to the sweet substance is not particularly limited as long as the amount can improve the quality of sweetness of the sweet substance, in particular, the body taste. Specific amount of the compound of the present invention is, for example, 0.000001% to 99.9999% by weight, preferably 0.00001% to 99.999% by weight, and more preferably 0.0001% to 99.99% by weight with respect to the high intensity sweetener.

It should be noted that the above description is a description about the mixture proportion with respect to the high intensity sweetener, and the use amount of the compound of the present invention in a food or a beverage is 1 ppb to 99.9% by weight, preferably 10 ppb to 10% by weight, and more preferably 1 ppm to 1% by weight.

[0048]

The sweetener of the present invention contains the sweet substance and the compound to be used in the present invention. The compound can be used alone or as a mixture of two or more kinds.

As the sweet substance, a high intensity sweetener is preferably used, and examples thereof include aspartame, sucralose, acesulfame K, neotame, ANS9801, saccharin, stevia, glycyrrhizin, thaumatin, monatin and monellin. Of those, aspartame, sucralose and acesulfame K are preferably used.

The sweet substance can be used alone or as a mixture of two or more kinds. It is known that the mixture of multiple sweet substances can improve the body taste of each of the sweet substances. Even in such a case, the combined use of the peptide or the amino acid to be used in the present invention with the sweet substances can further improve the body taste. Examples of the preferred combination of the sweet substances include a combination of two kinds optionally selected from aspartame, sucralose and acesulfame-K, or a combination of those three kinds.

Further, an example of the preferred combination includes a combination of aspartame and γ -Glu-Val-Gly

which is considered to have a high effect in the present invention. Specifically, these can be mixed so that the weight proportion of γ -Glu-Val-Gly might be 0.1 to 500 parts by weight with respect to 100 parts by weight of aspartame. As a matter of course, this is merely one example of mixture examples, and there is no need to be restricted to the above-mentioned proportion.

The sweetener of the present invention can of course be mixed with common sweeteners such as sucrose, glucose, fructose and sugar alcohols (for example, erythritol and maltitol). Examples of the form of the sweetener of the present invention include a powder, a granule and a liquid, and the physical properties are not limited.

Examples

[0049]

Hereinafter, the present invention is more specifically described with reference to examples. However, the scope of the present invention is not limited to those examples.

[0050]

[Reference Example 1] Preparation of calcium receptor gene (cRNA)

The gene of the calcium receptor was prepared as follows. On the basis of the DNA sequence registered at NCBI (calcium receptor: NM_000388), synthetic oligo DNAs (forward primer (SEQ ID NO: 1) and reverse primer (SEQ ID NO: 2)) used for PCR were synthesized.

[0051]

PCR was performed by using the primers and Pfu ultra DNA Polymerase (manufactured by Stratagene) under the following conditions by using human kidney cDNA (manufactured by Clontech) as a material. After a reaction at 94°C for 3 minutes, a cycle of reactions at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 2 minutes was repeated 35 times, and then a reaction was performed at 72°C for 7 minutes. Whether amplification was attained by PCR was confirmed by performing agarose electrophoresis of DNA, staining the DNA with a DNA staining reagent, and subsequent ultraviolet irradiation. The chain lengths of the PCR products were confirmed

by comparison with DNA markers of known sizes simultaneously subjected to the electrophoresis. The plasmid vector pBR322 was digested with the restriction enzyme EcoRV (manufactured by Takara). The gene fragment amplified by PCR was ligated to the cleavage site of the plasmid by using Ligation Kit (manufactured by Promega). The *Escherichia coli* DH5 α strain was transformed with each ligation reaction solution, and a transformant harboring the plasmid in which the PCR amplification product was cloned was selected. The PCR amplification product was confirmed by DNA base sequence analysis. By using the recombinant plasmid as a template together with a cRNA preparation kit (manufactured by Ambion), cRNA of the calcium receptor gene was prepared.

[0052]

[Reference Example 2] Preparation of various samples

As L-amino acid sample, 23 kinds of special grade amino acids including alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, ornithine and taurine (all of those from Ajinomoto Co., Inc.), and hydroxyproline (Nacalai Tesque, Inc.), were used. As D-Cys and D-Trp (Nacalai Tesque, Inc.) and calcium chloride, those of special grade were used.

[0053]

Further, as peptide sample, γ -Glu-Cys-Gly (Sigma Aldrich Japan K.K.), γ -Glu-Cys(SNO)-Gly (Dojindo Laboratories), γ -Glu-Ala (Bachem Feinchemikalien AG), γ -Glu-Gly (Bachem Feinchemikalien AG), γ -Glu-Cys (Sigma Aldrich Japan K.K.), γ -Glu-Met (Bachem Feinchemikalien AG), γ -Glu-Abu-Gly (Abu: α -aminobutyric acid, Bachem Feinchemikalien AG), γ -Glu-Thr (Kokusan Chemical Co., Ltd.), γ -Glu-Val (Kokusan Chemical Co., Ltd.), γ -Glu-Leu (custom synthesis product), γ -Glu-Ile (custom synthesis product), γ -Glu-Orn (Kokusan Chemical Co., Ltd.), Asp-Gly (custom synthesis product), Cys-Gly (custom synthesis product), Cys-Met (custom synthesis product), Glu-Cys (custom synthesis product), Gly-Cys (custom synthesis

product), Leu-Asp (custom synthesis product), γ -Glu-Val-Val (custom synthesis product), γ -Glu-Val-Glu (custom synthesis product), γ -Glu-Val-Lys (custom synthesis product), γ -Glu- γ -Glu-Val (custom synthesis product), γ -Glu-Gly-Gly (custom synthesis product), γ -Glu-Val-Phe (custom synthesis product), γ -Glu-Val-Ser (custom synthesis product), γ -Glu-Val-Pro (custom synthesis product), γ -Glu-Val-Arg (custom synthesis product), γ -Glu-Val-Asp (custom synthesis product), γ -Glu-Val-Met (custom synthesis product), γ -Glu-Val-Thr (custom synthesis product), γ -Glu-Val-His (custom synthesis product), γ -Glu-Val-Asn (custom synthesis product), γ -Glu-Val-Gln (custom synthesis product), γ -Glu-Val-Cys (custom synthesis product), γ -Glu-Val-Orn (custom synthesis product) and γ -Glu-Ser-Gly (custom synthesis product) were used. Glutamine and cysteine were prepared upon use, and the other samples were stored at -20°C after preparation. The peptides having a purity of 90% or higher were used. As only for γ -Glu-Cys, one having a purity of 80% or higher was used.

[0054]

After dissolving each sample in solution, pH of the solution showing an acidic or alkaline pH was adjusted to an approximately neutral pH by using NaOH or HCl. The composition of the solution used for dissolution of amino acids and peptides, and the solution used for preparation of *Xenopus laevis* oocytes and culture of the oocytes is as follows. 96 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , 5 mM Hepes, and pH 7.2.

[0055]

[Reference Example 3] Synthesis of γ -Glu-Val-Gly

Boc-Val-OH (8.69 g, 40.0 mmol) and Gly-OBzl $\cdot$ HCl (8.07 g, 40.0 mmol) were dissolved in methylene chloride (100 ml), and the solution was kept at 0°C . Triethylamine (6.13 ml, 44.0 mmol), HOBt (1-hydroxybenzotriazole, 6.74 g, 44.0 mmol) and WSC $\cdot$ HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 8.44 g, 44.0 mmol) were added to the solution, and the mixture was stirred overnight at room temperature. The reaction solution was

concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (200 ml). The solution was washed with water (50 ml), 5% of citric acid aqueous solution (50 ml × twice), saturated brine (50 ml), 5% of sodium hydrogen carbonate aqueous solution (50 ml × twice), and saturated brine (50 ml). The organic layer was dried over anhydrous magnesium sulfate, magnesium sulfate was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from ethyl acetate/n-hexane to obtain Boc-Val-Gly-OBzl (13.2 g, 36.2 mmol) as a white crystal.

[0056]

Boc-Val-Gly-OBzl (5.47 g, 15.0 mmol) was added to 4N HCl/dioxane solution (40 ml), and the mixture was stirred at room temperature for 50 minutes. Dioxane was removed by concentration under reduced pressure, n-hexane (30 ml) was added to the residue, and the mixture was concentrated under reduced pressure. The procedure was repeated 3 times to quantitatively obtain H-Val-Gly-OBzl·HCl.

H-Val-Gly-OBzl·HCl and Z-Glu-OBzl (5.57 g, 15.0 mmol) described above was dissolved in methylene chloride (50 ml), and the solution was kept at 0°C. Triethylamine (2.30 ml, 16.5 mmol), HOBt (1-hydroxybenzotriazole, 2.53 g, 16.5 mmol) and WSC·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 3.16 g, 16.5 mmol) were added to the solution, and the mixture was stirred at room temperature overnight for 2 days. The reaction solution was concentrated under reduced pressure, and the residue was dissolved in heated ethyl acetate (1,500 ml). The solution was washed with water (200 ml), 5% of citric acid aqueous solution (200 ml × twice), saturated brine (150 ml), 5% sodium hydrogen carbonate aqueous solution (200 ml × twice) and saturated brine (150 ml). The organic layer was dried over anhydrous magnesium sulfate, magnesium sulfate was removed by filtration, and the filtrate was concentrated under reduced pressure. The precipitated crystal was collected by filtration and dried under reduced pressure to obtain

Z-Glu(Val-Gly-OBzl)-OBzl (6.51 g, 10.5 mmol) as a white crystal.
[0057]

Z-Glu(Val-Gly-OBzl)-OBzl (6.20 g, 10.03 mmol) described above was suspended in ethanol (200 ml), 10% palladium on carbon (1.50 g) was added to the suspension, and a reduction reaction was performed under a hydrogen atmosphere at 55°C for 5 hours. During the reaction, 100 ml in a total volume of water were gradually added. The catalyst was removed by filtration using a Kiriya funnel, and the filtrate was concentrated under reduced pressure to a half volume. The reaction solution was further filtered through a membrane filter, and the filtrate was concentrated under reduced pressure. The residue was dissolved in a small volume of water, and to the solution, ethanol was added to precipitate a crystal, and the crystal was collected by filtration and dried under reduced pressure to obtain γ -Glu-Val-Gly as a white powder (2.85 g, 9.40 mmol).

[0058]

ESI-MS: $(M+H)^+ = 304.1$.

$^1\text{H-NMR}$ (400 MHz, D_2O) δ (ppm): 0.87 (3H, d, $J=6.8$ Hz), 0.88 (3H, d, $J=6.8$ Hz), 1.99-2.09 (3H, m), 2.38-2.51 (2H, m) 3.72 (1H, t, $J=6.35$ Hz), 3.86 (1H, d, $J=17.8$ Hz), 3.80 (1H, d, $J=17.8$ Hz), 4.07 (1H, d, $J=6.8$ Hz).

[0059]

[Reference Example 4] Synthesis of γ -Glu-Cys(S-Me)-Gly [Cys(S-Me): S-methylcysteine]

Reduced glutathione (15.0 g, 48.8 mmol) was added to water (45 ml), and sodium hydroxide (4.52 g, 2.2 equivalents, 107 mmol) was added portionwise to the mixture while the mixture was bubbled with nitrogen. Methyl iodide (4.56 ml, 1.5 equivalents, 73 mmol) was added to the mixture, and the solution was sealed and stirred at room temperature for 2 hours. The reaction solution was adjusted to pH 2 to 3 with concentrated hydrochloric acid, and ethanol (150 ml) was added to the reaction solution, and stored overnight in a refrigerator. A separated oily product from the supernatant was removed. When the remained oily product was dissolved in water,

and ethanol was gradually added to the solution, a partially crystallized oily product was precipitated. The supernatant was removed again. The residue was dissolved in water (300 ml), adsorbed to a column filled with an ion exchange resin (Dowex 1-acetate, 400 ml), washed with water, and then eluted with a 1N acetic acid aqueous solution. The eluate was concentrated under reduced pressure, and reprecipitated from water/ethanol to obtain γ -Glu-Cys(S-Me)-Gly as a white powder (5.08 g, 15.8 mmol).

[0060]

FAB-MS: $(M+H)^+ = 322$.

$^1\text{H-NMR}$ (400 MHz, D_2O) δ (ppm): 2.14 (3H, s), 2.15-2.22 (2H, m), 2.50-2.58 (2H, m), 2.86 (1H, dd, $J=9.0$ Hz, $J=14.0$ Hz), 3.03 (1H, dd, $J=5.0$ Hz, $J=14.0$ Hz), 3.84 (1H, t, $J=6.5$ Hz), 3.99 (2H, s), 4.59 (1H, dd, $J=5.0$ Hz, $J=9.0$ Hz)

[0061]

[Reference Example 5] Synthesis of other peptides

γ -Glu-Met(O), γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-t-Leu, γ -Glu-Cys(S-allyl)-Gly and γ -Glu-Cys(S-Me) were synthesized in accordance with Examples 3 and 4.

[0062]

[Reference Example 6] Evaluation of a calcium receptor-activating effect

For evaluation of the calcium receptor-activating effect, Calcium concentration-dependent Cl ionic current measuring method using a *Xenopus laevis* oocyte expression system was used. If activator is added to *Xenopus laevis* oocytes in which the calcium receptor is expressed, intracellular Calcium ions increase. Then, the Calcium concentration-dependent Cl channel opens, and the intracellular current value changes as an ionic current. By measuring the change in the intracellular current value, whether the calcium receptor-activating effect is present or not can be confirmed.

[0063]

Specifically, abdomen of *Xenopus laevis* was opened, and an

egg batch was taken out and then treated with a 1% collagenase solution at 20°C for 2 hours to obtain individual oocytes. Into each oocyte, 50 nl of 1 µg/µl receptor cRNA or 50 nl of sterilized water per oocyte were injected by using a micro glass capillary, and the oocytes were cultured at 18°C for 2 to 3 days. For the culture, a solution obtained by adding 2 mM of pyruvic acid, 10 U/ml of penicillin and 10 µg/ml of streptomycin to the solution mentioned in Reference Example 2 was used. After the culture, a test solution was added to the oocytes injected with cRNA or sterilized water. Electrophysiological measurement was performed by using an amplifier Geneclamp 500 (manufactured by Axon) and recording software AxoScope 9.0 (manufactured by Axon). The oocytes were voltage-clamped at -70 mV by the double electrode voltage clamp method, and the intracellular current via the Ca ion concentration-dependent Cl ion was measured. The maximum value of the intracellular current was considered as the response current value.

[0064]

[Reference Example 7] Evaluation of effect of calcium for activating calcium receptor

The calcium receptor-activating effect was evaluated by using the method described in Reference Example 6. That is, oocytes injected with cRNA of the calcium receptor or sterilized water was prepared, and voltage-clamped at -70 mV by the double electrode voltage clamp method. To the voltage-clamped oocytes, calcium was added (2 mM, 5 mM, 10 mM and 20 mM), and then Ca ion concentration-dependent Cl response current was measured. FIG. 1 shows the results. From the results, it was confirmed that cRNA of the calcium receptor injected into the oocytes was functionally expressed. Further, because the oocytes injected with water did not respond to even a high concentration of calcium, it was confirmed that the calcium receptor was not expressed in the oocytes themselves.

[0065]

[Reference Example 8] Evaluation of effect of L-amino acids for activating calcium receptor

The effect of L-amino acids for activating calcium receptor was evaluated by using the method described in Reference Example 6. That is, oocytes injected with cRNA of the calcium receptor or sterilized water were prepared, and voltage-clamped at -70 mV by the double electrode voltage clamp method. To the voltage-clamped oocytes, alanine (10 mM), arginine (10 mM), asparagine (10 mM), aspartic acid (10 mM), cysteine (10 mM), glutamine (10 mM), glutamic acid (10 mM), glycine (10 mM), histidine (10 mM), isoleucine (10 mM), leucine (10 mM), lysine (10 mM), methionine (10 mM), phenylalanine (10 mM), proline (10 mM), serine (10 mM), threonine (10 mM), tryptophan (10 mM), tyrosine (10 mM), valine (10 mM), ornithine (10 mM), taurine (10 mM) or hydroxyproline (10 mM) was added, and Ca ion concentration-dependent Cl response current was measured. FIG. 2 shows the results. From the results, it is demonstrated that cysteine, histidine, phenylalanine, tryptophan and tyrosine each had a definite calcium receptor-activating effect. The activating effect of the above-mentioned amino acids was reported in Proc. Natl. Acad. Sci. USA, Apr. 25, 2000, 97(9): 4814-9.

[0066]

[Reference Example 9] Evaluation of effect of D-cysteine for activating calcium receptor

The effect of D-cysteine for activating calcium receptor was evaluated by using the method described in Reference Example 6. That is, oocytes injected with cRNA of the calcium receptor or sterilized water were prepared, and voltage-clamped at -70 mV by the double electrode voltage clamp method. To the voltage-clamped oocytes, D-cysteine (10 mM), L-cysteine (10 mM), D-tryptophan (10 mM) or L-tryptophan (10 mM) was added, and Ca ion concentration-dependent Cl response current was measured. FIG. 3 shows the results. From the results, it is demonstrated that D-cysteine had a definite calcium receptor-activating effect.

[0067]

[Reference Example 10] Evaluation of effect of peptide for activating calcium receptor

The effect of peptide for activating calcium receptor was evaluated by using the method described in Reference Example 6. That is, oocytes injected with cRNA of the calcium receptor or sterilized water were prepared, and voltage-clamped at -70 mV by the double electrode voltage clamp method. To the voltage-clamped oocytes, γ -Glu-Cys-Gly (50 μ M), γ -Glu-Cys(SNO)-Gly (50 μ M), γ -Glu-Ala (50 μ M), γ -Glu-Gly (500 μ M), γ -Glu-Cys (50 μ M), γ -Glu-Met (500 μ M), γ -Glu-Thr (50 μ M), γ -Glu-Val (50 μ M), γ -Glu-Orn (500 μ M), Asp-Gly (1 mM), Cys-Gly (1 mM), Cys-Met (1 mM), Glu-Cys (50 μ M), Gly-Cys (500 μ M) or Leu-Asp (1 mM) was added, and Ca ion concentration-dependent Cl response current was measured. FIG. 4 shows the results. From the results, it is demonstrated that the above-mentioned peptide had a definite effect for activating calcium receptor.

[0068]

[Reference Example 11] Evaluation of effect of peptide for activating calcium receptor

The effect of peptide for activating calcium receptor was evaluated in the same manner as that of Reference Example 10. Each of the peptides shown in Table 1 was added to voltage-clamped oocytes at 1,000 μ M, 300 μ M, 100 μ M, 30 μ M, 10 μ M, 3 μ M, 1 μ M, 0.3 μ M and 0.1 μ M, and Ca ion concentration-dependent Cl response current was measured. The lowest concentration with which current was detected was shown in Table 1 as the activity. From the results, it is revealed that those 32 kinds of peptides each had a calcium receptor-activating effect.

[0069]

Table 1

Number	Peptide	Activity
1	γ -Glu-Met (O)	1,000 μ M
2	γ -Glu-Val-Val	1,000 μ M
3	γ -Glu-Val-Glu	1,000 μ M
4	γ -Glu-Val-Lys	1,000 μ M
5	γ -Glu-Val-Arg	1,000 μ M
6	γ -Glu-Val-Asp	1,000 μ M
7	γ -Glu-Val-Met	1,000 μ M
8	γ -Glu-Val-Thr	1,000 μ M
9	γ -Glu- γ -Glu-Val	1,000 μ M
10	γ -Glu-Val-NH ₂	1,000 μ M
11	γ -Glu-Val-ol	1,000 μ M
12	γ -Glu-Ser	300 μ M
13	γ -Glu-Tau	300 μ M
14	γ -Glu-Cys (S-Me) (O)	300 μ M
15	γ -Glu-Val-His	100 μ M
16	γ -Glu-Val-Orn	100 μ M
17	γ -Glu-Leu	100 μ M
18	γ -Glu-Ile	100 μ M
19	γ -Glu-t-Leu	100 μ M
20	γ -Glu-Cys (S-allyl) -Gly	100 μ M
21	γ -Glu-Val-Asn	30 μ M
22	γ -Glu-Gly-Gly	30 μ M
23	γ -Glu-Val-Phe	30 μ M
24	γ -Glu-Val-Ser	30 μ M
25	γ -Glu-Val-Pro	30 μ M
26	γ -Glu-Ser-Gly	30 μ M
27	γ -Glu-Cys (S-Me)	30 μ M
28	γ -Glu-Val-Cys	10 μ M
29	γ -Glu-Val-Gln	10 μ M
30	γ -Glu-Abu-Gly	3 μ M
31	γ -Glu-Cys (S-Me) -Gly	3 μ M
32	γ -Glu-Val-Gly	0.1 μ M

[0070]

[Reference Example 12] Kokumi-imparting activity of peptide and amino acid to be used in present invention

Typical examples are selected from γ -Glu-X-Gly where X represents Cys(SNO), Cys(S-allyl), Gly, Cys(S-Me), Abu or Ser, γ -Glu-Val-Y where Y represents Gly, Val, Glu, Lys, Phe, Ser, Pro, Arg, Asp, Met, Thr, His, Orn, Asn, Cys or Gln, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly,

Cys-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me), in each of which a calcium receptor-activating effect was found, and the presence or absence of the kokumi-imparting activity was examined by a sensory evaluation test.

[0071]

The sensory evaluation test was performed as follows. To distilled water containing sodium glutamate (0.05 g/dl), inosinic acid monophosphate (0.05 g/dl) and calcium chloride (1 mM), each of alliin (S-allyl-cysteine sulfoxide: control experiment of kokumi-imparting activity), γ -Glu-Cys-Gly, γ -Glu-Cys, γ -Glu-Ala and γ -Glu-Val was mixed as a sample in an amount of 0.2 g/dl, and the presence or absence of the kokumi-imparting activity was determined. As for a sample which is acidic when the sample is dissolved, pH of the sample was adjusted with NaOH to pH 6.8 to 7.2 before use. Table 2 shows the results.

[0072]

Table 2 Kokumi-imparting activity of calcium receptor promoter

Calcium receptor promoter	Kokumi-imparting activity
γ -Glu-Cys-Gly	+
γ -Glu-Cys	+
γ -Glu-Ala	+
γ -Glu-Val	+

[0073]

[Reference Example 13] Kokumi-imparting activity of peptide to be used in the present invention

A peptide in which a calcium receptor-activating effect was found was examined for intensity of its kokumi-imparting activity by a quantitative sensory evaluation test.

The quantitative sensory evaluation test was performed as follows. To distilled water containing sodium glutamate (0.05 g/dl), inosinic acid monophosphate (0.05 g/dl) and sodium chloride (0.5 g/dl), each of γ -Glu-Cys-Gly (glutathione), γ -Glu-Ala, γ -Glu-Met

and γ -Glu-Val was mixed as a sample in an amount of 0.1 g/dl, and the intensity of kokumi-imparting activity was measured. As for a sample which is acidic when the sample is dissolved, pH of the sample was adjusted with NaOH to pH 6.8 to 7.2 before use. Because it is known that glutathione can impart kokumi to a food, and thus, glutathione was used as a comparative control. The test was performed with n=3 based on the following sensory evaluation scores: control : 0 point and glutathione addition : 3 points. Table 3 shows the results. It should be noted that the term "initial and middle taste" is a collective term of an initial taste and a middle taste.

[0074]

Table 3

Sample	Concentration (g/dl)	Kokumi intensity		Taste profile
		Initial and middle taste	Aftertaste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.1	3.0	3.0	Thickness, growth, and continuity are enhanced.
γ -Glu-Ala	0.1	0.5	0.2	The effect is weak, but thickness is slightly enhanced.
γ -Glu-Met	0.1	1.5	0.4	Thickness and growth are slightly enhanced.
γ -Glu-Val	0.1	3.0	1.0	Thickness and growth are enhanced mainly in initial and middle taste.

[0075]

[Reference Example 14] Kokumi-imparting activity of peptide to be used in the present invention

A peptide in which a calcium receptor-activating effect was found was examined for intensity of its kokumi-imparting activity by a quantitative sensory evaluation test.

The quantitative sensory evaluation test was performed as follows. To distilled water containing sodium glutamate (0.05 g/dl), inosinic acid monophosphate (0.05 g/dl) and sodium chloride (0.5 g/dl), each of γ -Glu-Cys-Gly (glutathione), γ -Glu-Cys, γ -Glu-Val and γ -Glu-Val-Gly was mixed as a sample in an amount of 0.1 g/dl, or 0.01 g/dl as required, and the intensity of kokumi-imparting activity was measured. As for a sample which is acidic when the

sample is dissolved, pH of the sample was adjusted with NaOH to pH 6.8 to 7.2 before use. The test was performed with n=5 based on the following sensory evaluation scores: control : 0 point) and glutathione addition : 3 points. Table 4 shows the results.

[0076]

Table 4

Sample	Concentration (g/dl)	Kokumi intensity		Taste profile
		Initial and middle taste	Aftertaste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.1	3.0	3.0	Thickness, growth, and continuity are enhanced.
γ -Glu-Cys	0.1	2.0	2.0	A slightly weaker but comparable effect is exhibited compared with γ -Glu-Cys-Gly.
γ -Glu-Val	0.1	3.0	1.0	Thickness and growth are enhanced mainly in initial and middle taste.
γ -Glu-Val-Gly	0.1	*	*	*
γ -Glu-Val-Gly	0.01	3.0	3.0	Thickness and continuity are mainly enhanced. The whole taste is enhanced.

* Unmeasurable: kokumi-imparting activity is too strong to measure by sensory evaluation.

[0077]

[Reference Example 15] Kokumi-imparting activity of peptide to be used in present invention

A peptide in which a calcium receptor-activating effect was found was examined for intensity of its kokumi-imparting activity by a quantitative sensory evaluation test.

[0078]

The quantitative sensory evaluation test was performed as follows. To distilled water containing sodium glutamate (0.05 g/dl), inosinic acid monophosphate (0.05 g/dl) and sodium chloride (0.5 g/dl), each of γ -Glu-Cys-Gly (glutathione), γ -Glu-Abu-Gly and γ -Glu-Val-Gly was mixed as a sample in an amount of 0.1 g/dl or 0.01 g/dl, and the intensity of kokumi-imparting activity was measured. As for a sample which is acidic when the sample is dissolved, pH of the sample was adjusted with NaOH to pH 6.8 to 7.2 before use. The test was performed with n=12 based on the following sensory

evaluation scores: control : 0 point and glutathione addition : 3 points. Table 5 shows the results.

[0079]

Table 5

Sample	Concentration (g/dl)	Kokumi intensity		Taste profile
		Initial and middle taste	Aftertaste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.1	3.0	3.0	Thickness, growth, and continuity are enhanced.
γ -Glu-Abu-Gly	0.01	3.0	2.0	Thickness and growth are enhanced mainly in initial and middle taste.
γ -Glu-Val-Gly	0.01	3.0	3.0	Thickness and continuity are mainly enhanced. The whole taste is enhanced.

[0080]

[Example 1] Activity of compound to be used in present invention on sweetness (I)

A peptide in which a calcium receptor-activating effect and a kokumi-imparting activity were found was examined for its activities (improvement of body taste and improvement of bitterness) on typical high intensity sweeteners by a quantitative sensory evaluation test.

[0081]

The quantitative sensory evaluation test was performed as follows. As for standard solutions of each sweeteners, the following compounds were used. The following standard solutions have been adjusted so as to have almost the same degree of sweetness as each other.

- i) Sucrose (5 g/dl)
- ii) APM (0.025 g/dl)
- iii) Sucralose (0.008 g/dl)
- iv) Ace-K (0.025g/dl)

It should be noted that an addition effect of the peptides on each high intensity sweeteners was evaluated with reference to sucrose.

[0082]

To distilled water containing each of the above-mentioned sweeteners, γ -Glu-Cys-Gly (glutathione), γ -Glu-Val-Gly or γ -Glu-Abu-Gly was mixed as a sample at concentrations of 0.0001 to 1g/dl, respectively, and then each degree of the improvement of body taste and the improvement of bitterness on the sweet taste was measured.

As for a sample which has stronger acidic properties than the standard solution not containing the sample when the sample is dissolved, pH of the sample solution was adjusted with NaOH to achieve a range of $\text{pH} \pm 0.2$ with respect to the pH of the standard solution before use.

[0083]

The sensory evaluation for the improvement of the body taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding glutathione and/or the peptide of the present invention) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The higher score than 0 point means that the sample has a body taste-improving effect.

On the other hand, the sensory evaluation for the improvement of the bitter taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding glutathione and/or the peptide of the present invention) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The lower score than 0 point means that the sample has a bitterness-improving effect.

As a result of the evaluation, activities were widely exhibited at the above-mentioned addition concentrations. Tables 6 to 8 each show the results at typical concentrations. As clear from the results shown in Tables 6, 7 and 8, it was confirmed that the peptides of the present invention each has a body taste-improving effect at

a lower concentration than glutathione. Further, it is also confirmed that the peptide of the present invention has a bitterness-improving effect. In general, the addition of the peptides provides the improvement of the body taste and the reduction in the bitter taste, and results in a sweet taste close to sucrose.

[0084]

Table 6

Sample ii) APM	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	-0.5	Sweet taste is wholly enhanced.
γ -Glu-Val-Gly	0.0001	0.5	-0.2	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.5	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Abu-Gly	0.001	0.7	-0.1	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.5	-0.1	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[0085]

Table 7

Sample iii) sucralose	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	0	Sweet taste with growth is enhanced in middle taste.
γ -Glu-Val-Gly	0.0001	0.7	-0.1	Outstanding sweet taste is harmonized in initial taste and aftertaste.
γ -Glu-Val-Gly	0.001	1.3	-0.2	Outstanding sweet taste is harmonized in initial taste and aftertaste.
γ -Glu-Abu-Gly	0.001	0.5	-0.1	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.0	-0.1	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[0086]

Table 8

Sample iv) Ace-K	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	-0.6	Sweet taste is enhanced in middle taste to aftertaste.
γ -Glu-Val-Gly	0.0001	0.7	-0.6	Sweet taste with growth is enhanced in middle taste.
γ -Glu-Val-Gly	0.001	1.2	-1.2	Sweet taste with growth is enhanced in middle taste.
γ -Glu-Abu-Gly	0.001	0.5	-0.6	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.1	-1.2	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[0087]

[Example 2] Activity of compound to be used in present invention on sweet taste (II)

A peptide in which a calcium receptor-activating effect and a kokumi-imparting activity were found was examined for its effects (improvement of body taste and improvement of bitterness) on products in which high intensity sweeteners are typically combined, by a quantitative sensory evaluation test.

[0088]

The quantitative sensory evaluation test was performed as follows. As for combination standard solution of each sweeteners, the following compounds were used. The following standard solutions have been adjusted so as to have almost the same degree of sweetness as each other.

i) Sucrose (5 g/dl)

ii) APM (0.02 g/dl)+Ace-K (0.005 g/dl) [APM:Ace-K=8:2 as a ratio of degrees of sweetness]

iii) Sucralose (0.0058 g/dl)+Ace-K (0.0075 g/dl)

[sucralose:Ace-K=7:3 as a ratio of degrees of sweetness]

iv) APM (0.0125 g/dl)+sucralose (0.0042 g/dl) [APM:sucralose=1:1 as a ratio of degrees of sweetness]

v) APM (0.0125 g/dl)+sucralose (0.0021 g/dl)+Ace-K (0.0063 g/dl)

[APM:sucralose:Ace-K=2:1:1 as a ratio of degrees of sweetness]

It should be noted that an addition effect of the peptides on each of the high intensity sweeteners was evaluated with reference to sucrose.

[0089]

To distilled water containing each of the sweeteners, γ -Glu-Cys-Gly (glutathione), γ -Glu-Val-Gly or γ -Glu-Abu-Gly was mixed as a sample at concentrations of 0.0001 to 1 g/dl, respectively, and then each degree of the improvement of the body taste and the improvement of the bitterness on the sweet taste was measured. The addition effect of the peptides on each of the high intensity sweeteners was evaluated with reference to sucrose.

As for a sample which has stronger acidic properties than the standard solution not containing the sample when the sample is dissolved, pH of the sample was adjusted with NaOH to achieve a range of $\text{pH} \pm 0.2$ with respect to the pH of the standard solution before use.

[0090]

The sensory evaluation for the improvement of the body taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding glutathione and/or the peptide of the present invention) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The higher score than 0 point means that the sample has a body taste-improving effect.

On the other hand, the sensory evaluation for the improvement of the bitter taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding glutathione and/or the peptide of the present invention) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The lower

score than 0 point means that the sample has a bitterness-improving effect.

As a result of the evaluation, activities were widely exhibited at the above-mentioned addition concentrations. Tables 9 to 12 each show the results at typical concentrations. As clear from the results shown in Tables 9 to 12, it was confirmed that the peptides of the present invention each has a body taste-improving effect at a lower concentration than glutathione. Further, it is also confirmed that the peptide of the present invention has a bitterness-improving effect. In general, the addition of the peptides provides the improvement of the body taste and the reduction in the bitter taste, and results in a sweet taste close to sucrose.

[0091]

Table 9

Sample ii) APM: Ace-K =8:2	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	-0.3	Sweet taste is wholly enhanced.
γ -Glu-Val-Gly	0.0001	0.6	-0.3	Sweet taste with growth is enhanced in middle taste. Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Abu-Gly	0.001	0.6	-0.3	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.3	-0.7	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[0092]

Table 10

Sample iii) sucralose: Ace-K =7:3	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	-0.3	Sweet taste is enhanced in middle taste.
γ -Glu-Val-Gly	0.0001	0.7	-0.3	Harmony is wholly present. Heavy taste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Harmony is wholly present. Heavy taste is suppressed and sharpness is improved.
γ -Glu-Abu-Gly	0.001	0.5	-0.3	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.1	-0.7	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[0093]

Table 11

Sample iv) APM:sucralose= 1:1	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.0	-0.3	Sweet taste is enhanced in middle taste.
γ -Glu-Val-Gly	0.0001	0.6	-0.2	Harmony is wholly present and strong taste is achieved.
γ -Glu-Val-Gly	0.001	1.3	-0.4	Harmony is wholly present and strong taste is achieved.
γ -Glu-Abu-Gly	0.001	0.6	-0.1	Harmony is present. Sweet taste is enhanced in initial taste and middle taste.
γ -Glu-Abu-Gly	0.01	1.3	-0.1	Harmony is present. Sweet taste is enhanced in initial taste and middle taste.

[0094]

Table 12

Sample v) APM:sucralose: Ace-K=2:1:1	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Cys-Gly	0.05	1.2	-0.3	Sweet taste is wholly enhanced.
γ -Glu-Val-Gly	0.0001	0.6	-0.3	Harmony is wholly present. Heavy taste is suppressed and sharpness in aftertaste is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Harmony is wholly present. Heavy taste is suppressed and sharpness in aftertaste is improved.
γ -Glu-Abu-Gly	0.001	0.6	-0.4	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Abu-Gly	0.01	1.3	-0.8	Sweet taste is enhanced in initial taste and middle taste. Sweet taste remaining in aftertaste is suppressed.

[Example 3] Activity of compound to be used in present invention on sweet taste (III)

A peptide in which a calcium receptor-activating effect and a kokumi-imparting activity were found, and cinacalcet which was known to have a calcium receptor-activating effect were examined for their activities (improvement of body taste and improvement of bitterness) on typical high intensity sweeteners by a quantitative sensory evaluation test.

[0095]

The quantitative sensory evaluation test was performed as

follows. As for standard solutions of each sweeteners, the following compounds were used. The following standard solutions have been adjusted so as to have almost the same degree of sweetness as each other.

- i) Sucrose (5 g/dl)
- ii) APM (0.025 g/dl)
- iii) Sucralose (0.008 g/dl)
- iv) Ace-K (0.025 g/dl)

It should be noted that an addition effect of the samples on each of the high intensity sweeteners was evaluated with reference to sucrose.

[0096]

To distilled water containing each sweeteners, γ -Glu-Val-Gly or cinacalcet was mixed as a sample at concentrations of 0.0001 to 1 g/dl, respectively, and then each degree of the improvement of the body taste and the improvement of the bitterness on the sweet taste was measured.

As for a sample which has stronger acidic properties than the standard solution not containing the sample when the sample is dissolved, pH of the sample was adjusted with NaOH to achieve a range of $\text{pH} \pm 0.2$ with respect to the pH of the standard solution before use.

[0097]

The sensory evaluation for the improvement of the body taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding a sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The higher score than 0 point means that the sample has a body taste-improving effect.

On the other hand, the sensory evaluation for the improvement of the bitter taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water

only and without adding a sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The lower score than 0 point means that the sample has a bitterness-improving effect.

As a result of the evaluation, activities were widely exhibited at the above-mentioned addition concentrations. Tables 13 to 15 each show the results at typical concentrations. As clear from the results shown in Tables 13 to 15, it is confirmed that γ -Glu-Val-Gly and cinacalcet each has a body taste-improving effect at almost the same concentration. Further, it is also confirmed that the sample of the present invention has a bitterness-improving effect. In general, the addition of the sample provides the improvement of the body taste and the reduction in the bitter taste, and results in sweet taste close to sucrose.

[0098]

Table 13

Sample ii) APM	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.5	-0.2	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.5	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
Cinacalcet	0.0001	0.8	-0.2	Sweet taste remaining in aftertaste is suppressed. Growth is present in middle taste to aftertaste.
Cinacalcet	0.001	1.5	-0.5	Sweet taste remaining in aftertaste is suppressed. Growth is present in middle taste to aftertaste.

[0099]

Table 14

Sample iii) sucralose	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.7	-0.1	Outstanding sweet taste is harmonized in initial taste and aftertaste.
γ -Glu-Val-Gly	0.001	1.3	-0.2	Outstanding sweet taste is harmonized in initial taste and aftertaste.
Cinacalcet	0.0001	0.5	-0.1	Outstanding sweet taste is harmonized in initial taste and aftertaste.
Cinacalcet	0.001	1.1	-0.2	Outstanding sweet taste is harmonized in

				initial taste and aftertaste.
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[0100]

Table 15

Sample iv) Ace-K	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.7	-0.6	Sweet tastewithgrowthis enhanced inmiddle taste.
γ -Glu-Val-Gly	0.001	1.2	-1.2	Sweet tastewithgrowthis enhanced inmiddle taste.
Cinacalcet	0.0001	0.6	-0.2	Sweet tastewithgrowthis enhanced inmiddle taste to aftertaste.
Cinacalcet	0.001	1.1	-0.3	Sweet tastewithgrowthis enhanced inmiddle taste to aftertaste.

[0101]

[Example 4] Activity of compound to be used in present invention on sweet taste (IV)

A peptide in which a calcium receptor-activating effect and a kokumi-imparting activity were found, and cinacalcet which was known to have a calcium receptor-activating effect were examined for their effects (improvement of body taste and improvement of bitter taste) on products in which high intensity sweeteners are typically combined, by a quantitative sensory evaluation test.

[0102]

The quantitative sensory evaluation test was performed as follows. As for combination standard solution of each sweeteners, the following compound was used. The following standard solutions have been adjusted so as to have almost the same degree of sweetness as each other.

- i) Sucrose (5 g/dl)
- ii) APM (0.02 g/dl)+Ace-K (0.005 g/dl) [APM:Ace-K=8:2 as a ratio of degrees of sweetness]
- iii) Sucralose (0.0058 g/dl)+Ace-K (0.0075 g/dl)
[sucralose:Ace-K=7:3 as a ratio of degrees of sweetness]
- iv) APM (0.0125 g/dl)+sucralose (0.0042 g/dl) [APM:sucralose=1:1 as a ratio of degrees of sweetness]

v) APM (0.0125 g/dl)+sucralose (0.0021 g/dl)+Ace-K (0.0063 g/dl)
[APM:sucralose:Ace-K=2:1:1 as a ratio of degrees of sweetness]

It should be noted that an addition effect of the peptides on each of the high intensity sweeteners was evaluated with reference to sucrose.

[0103]

To distilled water containing each sweeteners mentioned above, γ -Glu-Val-Gly or cinacalcet was mixed as a sample at concentrations of 0.0001 to 1 g/dl, respectively, and then each degree of the improvement of the body taste and the improvement of the bitter taste on the sweet taste was measured. As for a sample, the addition effect of the sample on each of the high intensity sweeteners was evaluated with reference to sucrose.

As for a sample which has stronger acidic properties than the standard solution not containing the sample when the sample is dissolved, pH of the sample was adjusted with NaOH to achieve a range of $\text{pH} \pm 0.2$ with respect to the pH of the standard solution before use.

[0104]

The sensory evaluation for the improvement of the body taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding the sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The higher score than 0 point means that the sample has a body taste-improving effect.

On the other hand, the sensory evaluation for the improvement of the bitter taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding the sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The lower score than 0 point means that the sample

has a bitterness-improving effect.

As a result of the evaluation, activities were widely exhibited at the above-mentioned addition concentrations. Tables 16 to 19 each show the results at typical concentrations. As clear from the results shown in Tables 16 to 19, it is confirmed that γ -Glu-Val-Gly and cinacalcet each has a body taste-improving effect at almost the same concentration. Further, it is also confirmed the sample of the present invention has a bitterness-improving effect. In general, the addition of the sample provides the improvement of the body taste and the reduction in the bitter taste, and results in a sweet taste close to sucrose.

[0105]

Table 16

Sample ii) APM:Ace-K =8:2	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.6	-0.3	Sweet taste with growth is enhanced in middle taste. Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Sweet taste remaining in aftertaste is suppressed and sharpness is improved.
Cinacalcet	0.0001	0.5	-0.1	Sweet taste with growth is enhanced in middle taste to aftertaste.
Cinacalcet	0.001	1.1	-0.3	Sweet taste with growth is enhanced in middle taste to aftertaste.

[0106]

Table 17

Sample iii) sucralose:Ace- K=7:3	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.7	-0.3	Harmony is wholly present. Heavy taste is suppressed and sharpness is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Harmony is wholly present. Heavy taste is suppressed and sharpness is improved.
Cinacalcet	0.0001	0.8	-0.1	Sweet taste with thickness and growth is enhanced in middle taste to aftertaste.
Cinacalcet	0.001	1.5	-0.3	Sweet taste with thickness and growth is enhanced in middle taste to aftertaste.

[0107]

Table 18

Sample iv) APM:sucralose= 1:1	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.6	-0.2	Harmony is wholly present and strong taste is achieved.
γ -Glu-Val-Gly	0.001	1.3	-0.4	Harmony is wholly present and strong taste is achieved.
Cinacalcet	0.0001	0.5	-0.2	Sweet taste with thickness and growth is enhanced in middle taste.
Cinacalcet	0.001	1.1	-0.2	Sweet taste with thickness and growth is enhanced in middle taste.

[0108]

Table 19

Sample v) APM:sucralose: Ace-K=2:1:1	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.6	-0.3	Harmony is wholly present. Heavy taste is suppressed and sharpness in aftertaste is improved.
γ -Glu-Val-Gly	0.001	1.3	-0.7	Harmony is wholly present. Heavy taste is suppressed and sharpness in aftertaste is improved.
Cinacalcet	0.0001	0.5	-0.2	Harmony and richness are present in middle taste to aftertaste.
Cinacalcet	0.001	1.1	-0.4	Harmony and richness are present in middle taste to aftertaste.

[Example 5] Activity of the compound to be used in present invention in drink yogurt containing high intensity sweetener

A peptide in which a calcium receptor-activating effect and a kokumi-imparting activity were found, and cinacalcet which was known to have a calcium receptor-activating effect were examined for their activities (improvement of body taste and improvement of bitter taste) in a drink yogurt containing a typical high intensity sweetener by a quantitative sensory evaluation test.

[0109]

The quantitative sensory evaluation test was performed as follows. As for a drink yogurt standard solution, the following compounds were used. The following standard solutions have been adjusted so as to have almost the same degree of sweetness as each

other.

- i) Sucrose (5 g/dl)
- ii) APM (0.025 g/dl)

It should be noted that an addition effect of the sample on each of the high intensity sweeteners was evaluated with reference to sucrose.

[0110]

To a drink yogurt containing each sweeteners, γ -Glu-Val-Gly or cinacalcet was mixed as a sample at concentrations 0.0001 to 1 g/dl, respectively, and then each degree of the improvement of the body taste and the improvement of the bitter taste on the sweet taste was measured.

As for a sample which has stronger acidic properties than the standard solution not containing the sample when the sample is dissolved, pH of the sample was adjusted with NaOH to achieve a range of $\text{pH} \pm 0.2$ with respect to the pH of the standard solution before use.

[0111]

The sensory evaluation for the improvement of the body taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding the sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The higher score than 0 point means that the sample has a body taste-improving effect.

On the other hand, the sensory evaluation for the improvement of the bitter taste was performed with $n=12$ on the basis of the following criteria: a control (obtained with adding distilled water only and without adding the sample) was defined as 0 point, and by using the control as a standard, weak: -2 points; slightly weak: -1 point; slightly strong: 1 point, and strong: 2 points were defined, respectively. The lower score than 0 point means that the sample has a bitterness-improving effect.

As a result of the evaluation, activities were widely exhibited at the above-mentioned addition concentrations. Table 20 shows the results at typical concentrations. As clear from the results shown in Table 20, it is confirmed that γ -Glu-Val-Gly and cinacalcet each has a body taste-improving effect at almost the same concentration. Further, it is also confirmed that the sample of the present invention has a bitterness-improving effect. In general, the addition of the sample provides the improvement of the body taste and the reduction in the bitter taste, and results in a sweet taste close to sucrose.

[0112]

Table 20

Sample ii) APM	Concentration (g/dl)	Taste		Taste profile
		Body taste	Bitter taste	
Control	-	0	0	-
γ -Glu-Val-Gly	0.0001	0.5	-0.2	Sweet taste remaining in aftertaste is suppressed.
γ -Glu-Val-Gly	0.001	1.4	-0.5	Growth is present in middle taste. Sweet taste remaining in aftertaste is suppressed.
Cinacalcet	0.0001	0.5	-0.2	Sweet taste remaining in aftertaste is suppressed.
Cinacalcet	0.001	1.6	-0.1	Thickness is present. Sweet taste remaining in aftertaste is suppressed.

Industrial Applicability

[0113]

The use of the sweetener, in particular, the high intensity sweetener such as aspartame in combination with the compound having a calcium receptor-activating effect, preferably the amino acid or the peptide, improves the body taste or the bitter taste of the high intensity sweetener such as aspartame. The sweetener of the present invention which has the improved body taste and bitter taste of the sweet taste can be widely utilized for general foods such as beverages and water ices.

Further, the compound having a calcium receptor-activating effect can be widely utilized as an additive agent capable of improving the body taste and the bitter taste of the sweetener, in particular,

the high intensity sweetener.

CLAIMS

1. A sweetener which comprises a sweet substance and a compound having calcium receptor-activating effect, wherein the compound comprises one or more kinds of amino acid or peptide selected from the group consisting of γ -Glu-X-Gly where X represents an amino acid other than Cys or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu, γ -Glu-Cys(S-Me), γ -Glu-Val-GlyA and γ -Glu-Val-LacA.

2. The sweetener according to claim 1, wherein X represents Cys(SNO), Cys(S-allyl), Gly, Cys(S-Me), α -aminobutyric acid(Abu), tert-leucine(t-Leu), cycloleucine(Cle), α -aminoisobutyric acid(Aib), L-penicillamine(Pen) or Ser, and Y represents Gly, Val, Glu, Lys, Phe, Ser, Pro, Arg, Asp, Met, Thr, His, Orn, Asn, Cys or Gln.

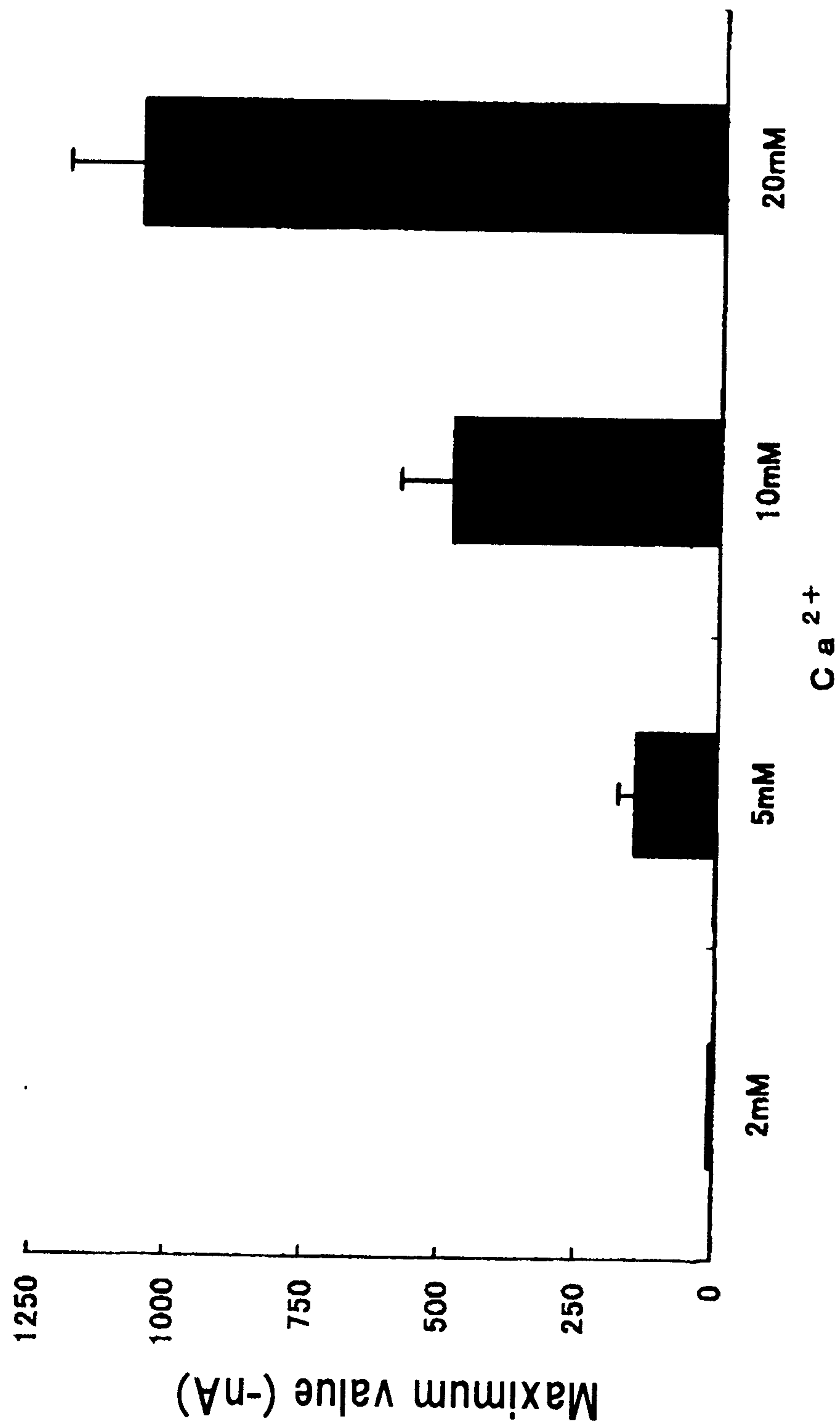
3. The sweetener according to claim 1 or 2, wherein the compound is selected from γ -Glu-Val-Gly and γ -Glu-Abu-Gly.

4. The sweetener according to any one of claims 1 to 3, wherein the sweet substance comprises one or more kinds of substance selected from the group consisting of aspartame, sucralose, acesulfame K, neotame, N-[N-[3-(3-hydroxy-4-methoxyphenyl)propyl]- α -aspartyl]-L-phenylalanine 1-methyl ester, saccharin, stevia, glycyrrhizin, thaumatin, monatin and monellin.

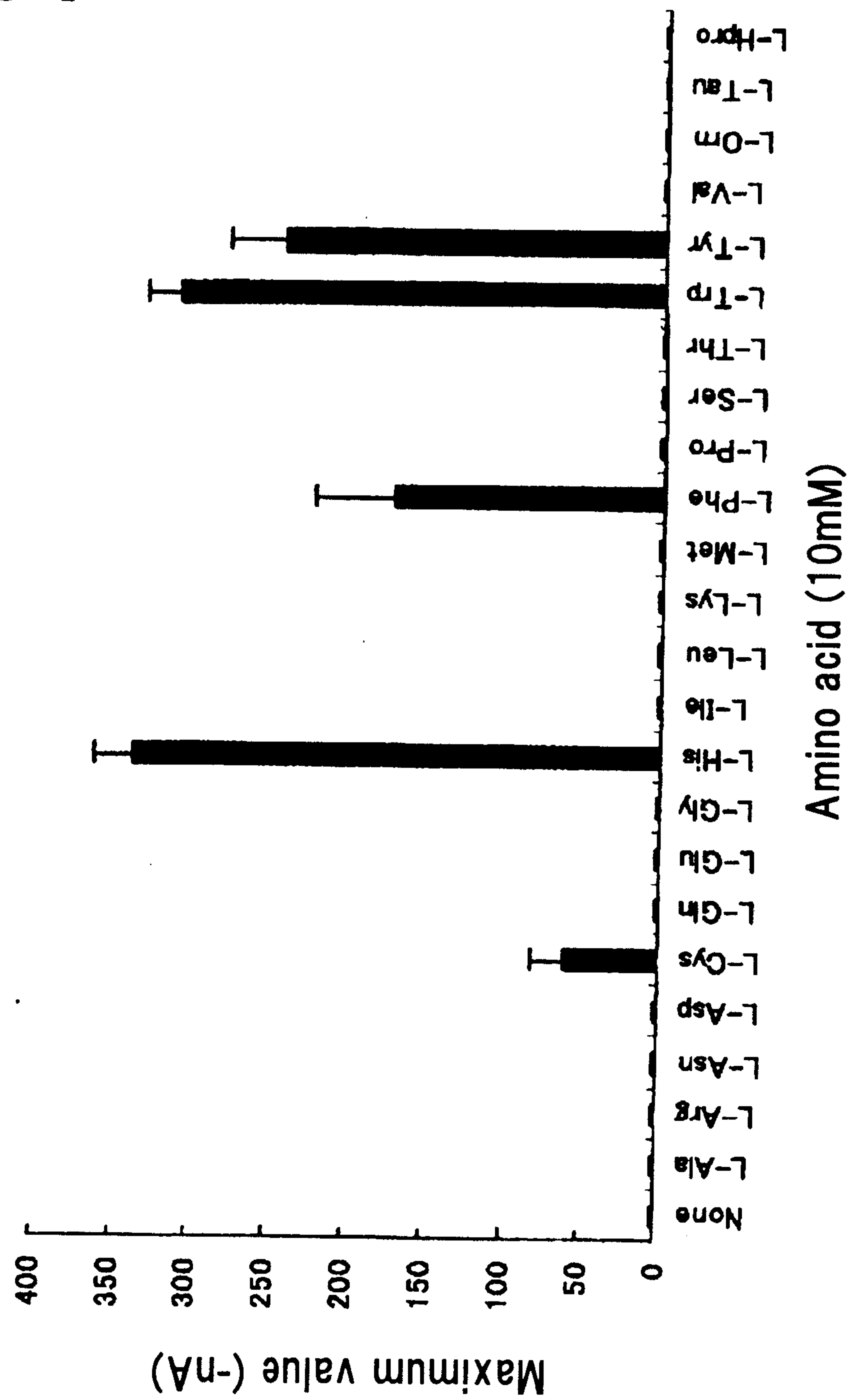
5. An additive agent to be added to a sweet substance for improving a body taste of the sweet substance, which comprises a compound having calcium receptor-activating effect, wherein the compound comprises one or more kinds of amino acid or peptide selected from the group consisting of γ -Glu-X-Gly where X represents an amino acid other than Cys or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me).

6. An additive agent to be added to a sweet substance for improving a bitter taste of the sweet substance, which comprises a compound having calcium receptor-activating effect, wherein the compound comprises one or more kinds of amino acid or peptide selected from the group consisting of γ -Glu-X-Gly where X represents an amino acid other than Cys or an amino acid derivative, γ -Glu-Val-Y where Y represents an amino acid or an amino acid derivative, γ -Glu-Ala, γ -Glu-Gly, γ -Glu-Cys, γ -Glu-Met, γ -Glu-Thr, γ -Glu-Val, γ -Glu-Orn, Asp-Gly, Cys-Met, Glu-Cys, Gly-Cys, Leu-Asp, D-Cys, γ -Glu-Met(O), γ -Glu- γ -Glu-Val, γ -Glu-Val-NH₂, γ -Glu-Val-ol, γ -Glu-Ser, γ -Glu-Tau, γ -Glu-Cys(S-Me)(O), γ -Glu-Leu, γ -Glu-Ile, γ -Glu-t-Leu and γ -Glu-Cys(S-Me).

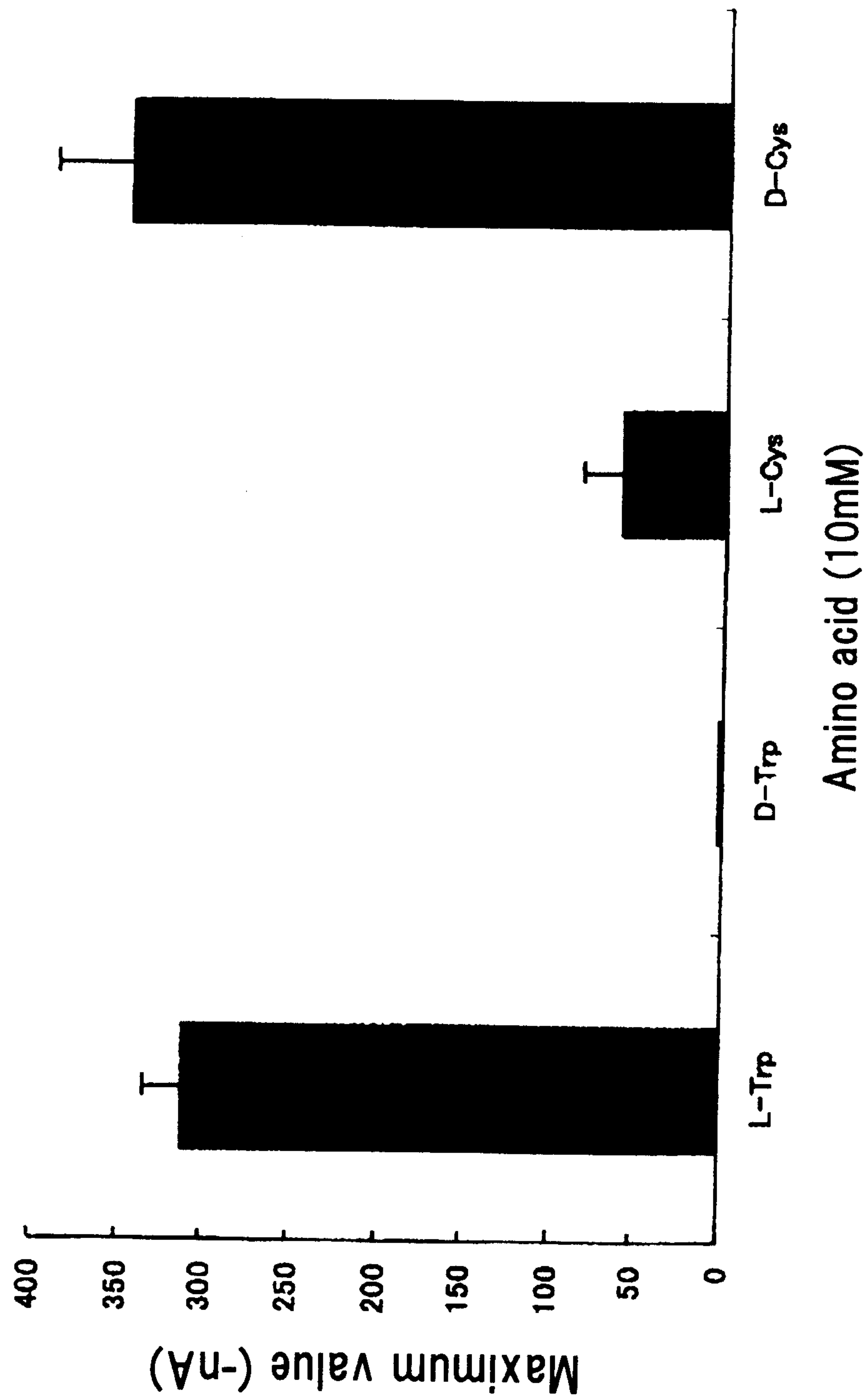
[Fig.1]



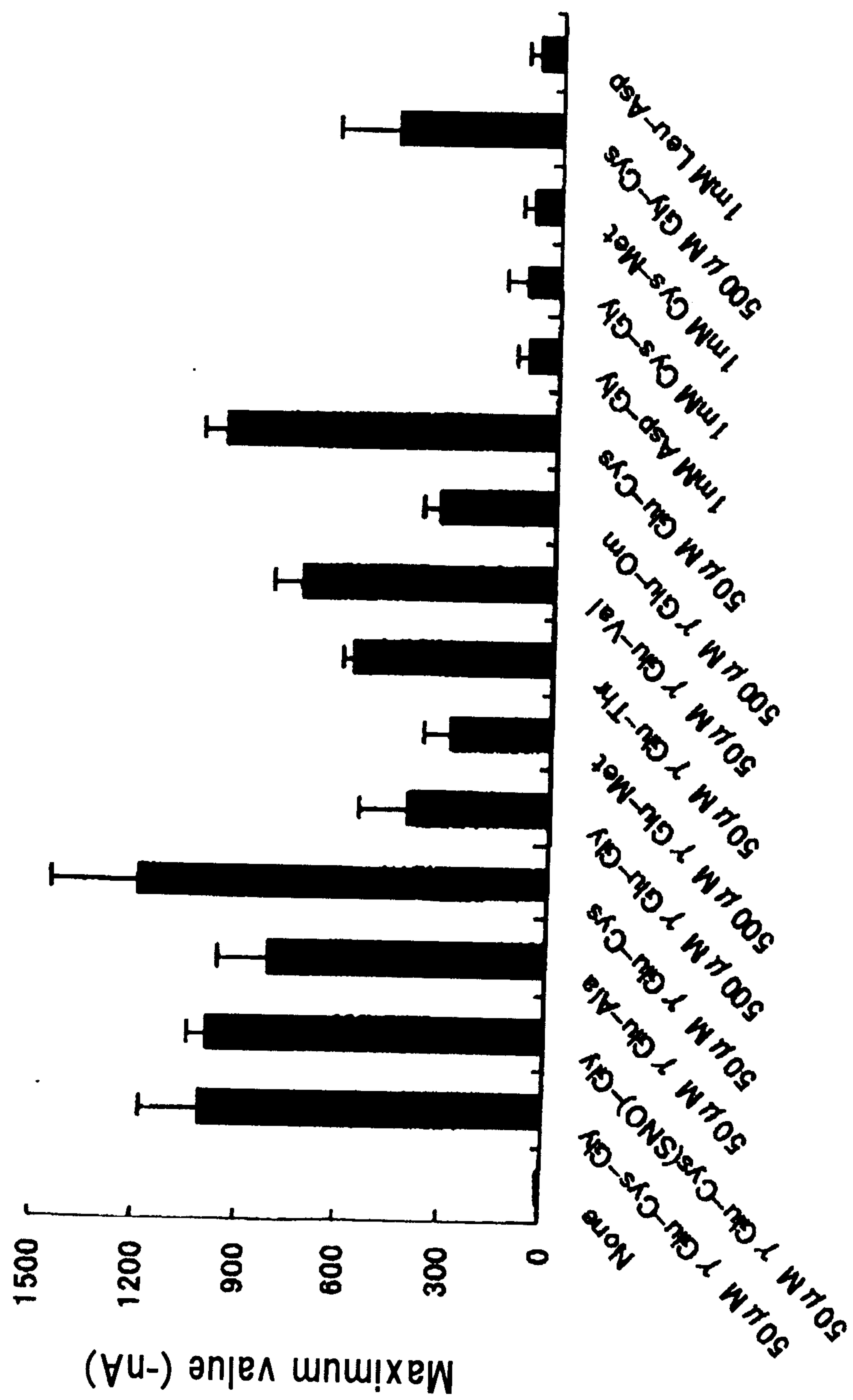
[Fig.2]



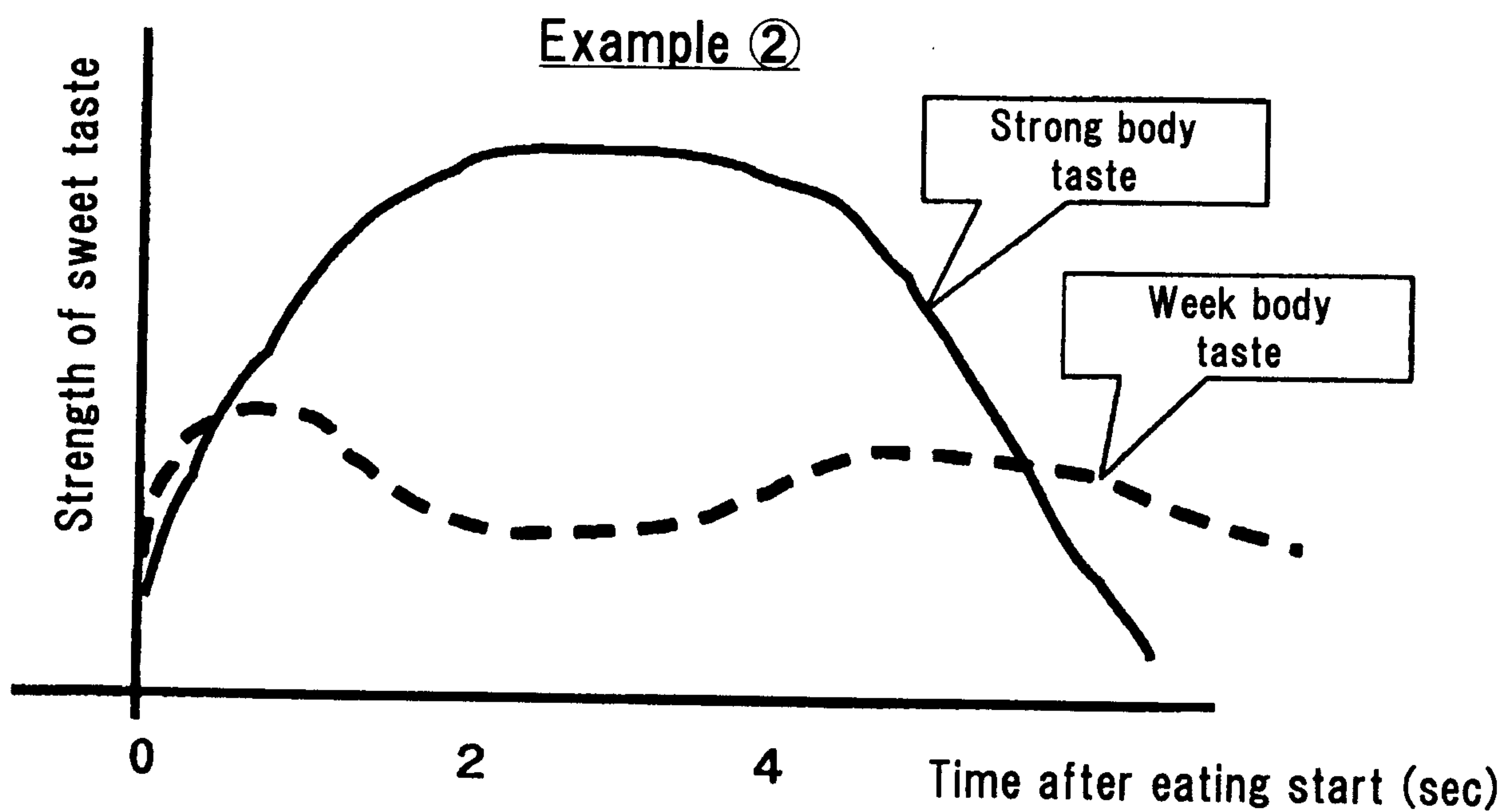
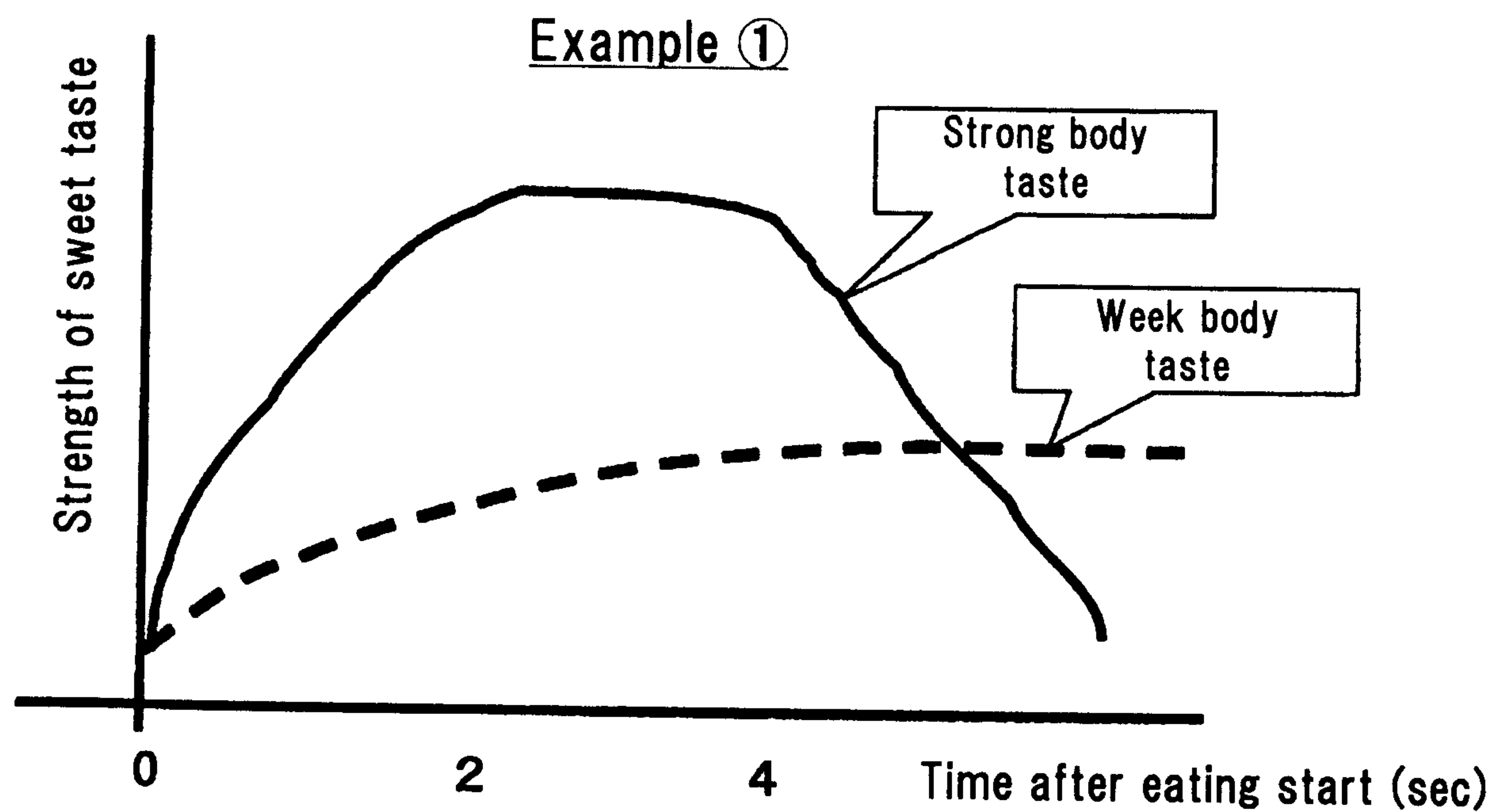
[Fig.3]



[Fig.4]



[Fig.5]



[Fig.5]

