CONVENTION

AUSTRALIA

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APPLICATION FOR A STANDARD PATENT

628514

Mitsui Norin Co., Ltd.
1-20, Nihonbashimuromachi 3-chome, Chuo-ku, Tokyo, JAPAN

hereby applies for the grant of a standard patent for an invention entitled:

INHIBITIVE AGENT AGAINST ACTIVITY OF -AMYLASE

which is described in the accompanying complete specification.

Details of basic application(s):-
270228/1989 JAPAN 19 October 1989

Address for Service:

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DATED this TWELFTH day of APRIL 1990

PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
Mitsui Norin Co., Ltd.

By: David P Fitzpatrick

Our Ref : 170384
POF Code: 1286/115611

6010

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DECLARATION FOR A PATENT APPLICATION

▼ INSTRUCTIONS
(a) Insert "Convention" if applicable
(b) Insert FULL name(s) of applicant(s)

In support of the (a) Convention application made by
(b) Mitsui Norin Co., Ltd.

(c) Insert "of addition" if applicable
(d) Insert TITLE of invention

(hereinafter called "applicant(s)" for a patent (c) for an invention entitled (d) INHIBITIVE AGENT AGAINST ACTIVITY OF α-AMYLASE

(e) Insert FULL name(s) AND address(es) of declarant(s) (See headnote*)

I/~~We~~ (e) KazuoTsumura on behalf of Mitsui Norin Co., Ltd. of 1-20, Nihonbashimuromachi 3-chome, Chuo-ku, Tokyo, JAPAN

do solemnly and sincerely declare as follows:

~~1. I am/We are the applicant(s).~~

(or, in the case of an application by a body corporate)

1. I am/~~We are~~ authorized to make this declaration on behalf of the applicant(~~s~~).

~~2. I am/We are the actual inventor(s) of the invention.~~

(or, where the applicant(s) is/are not the actual inventor(s))

(f) Insert FULL name(s) AND address(es) of actual inventor(s)

2. (f) Yukihiko Hara 2-7, Minamisurugadai 2-chome, Fujieda-shi, Shizuoka-ken, JAPAN.
Miwa Honda 7-23-A202, Seko 2-chome, Fijieda_shi, Shizuoka_ken, JAPAN

(g) Recite how applicant(s) derive(s) title from actual inventor(s) (See headnote**)

~~is~~/are the actual inventor(s) of the invention and the facts upon which the applicant(~~s~~) is/~~are~~ entitled to make the application are as follows:

(g) The applicant is the assignee of the invention from the said actual inventor(s)

(**Note:** *Paragraphs 3 and 4 apply* **only** *to Convention applications)*

(h) Insert country, filing date, and basic applicant(s) for the/or EACH basic application

3. The basic application(s) for patent or similar protection on which the application is based ~~is~~/are identified by **country, filing date, and basic applicant(s)** as follows:

(h) JAPAN
12 April 1990
Mitsui Norin Co., Ltd.

4. The basic application(~~s~~) referred to in paragraph 3 hereof was/~~were~~ the first application(~~s~~) made in a Convention country in respect of the invention the subject of the application.

(k) Insert PLACE of signing

(l) Insert DATE of signing

(m) Signature(s) of declarant(s)

Note: No legalization or other witness required

Declared at (k) Tokyo, Japan

Dated (l) May 15, 1990

(m) Kazuo Tsumura

Kazuo Tsumura/President
Mitsui Norin Co., Ltd.

To: The Commissioner of Patents

P18/7/78

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(12) PATENT ABRIDGMENT (11) Document No. AU-B-53195/90
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 628514

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INHIBITIVE AGENT AGAINST ACTIVITY OF ALPHA-AMYLASE

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(56) Prior Art Documents
AU 53194/90

(57) Claim

1. A method of inhibiting the activity of α-amylase in a patient in need thereof which comprises administering an effective amount of a tea polyphenol.

2. A method as claimed in claim 1 wherein the tea polyphenol is selected from the group comprising epigallocatechin gallate, epicatechin gallate, and the isomers thereof, free theaflavin, theaflavin monogallate A, theaflavin monogallate B and theaflavin digallate.

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628514

COMPLETE SPECIFICATION
(ORIGINAL)

Class Int. Class

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Lodged:

Complete Specification Lodged:
Accepted:
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Related Art:

Applicant(s):

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Complete Specification for the invention entitled:

INHIBITIVE AGENT AGAINST ACTIVITY OF α-AMYLASE

Our Ref : 170384
POF Code: 1286/115611

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

6006

INHIBITIVE AGENT AGAINST ACTIVITY OF α-AMYLASE

BACKGROUND OF THE INVENTION

The present invention relates to a novel inhibitive agent against the activity of α-amylase or, more particularly, to an inhibitive agent against the activity of α-amylase with high specificity in the reaction with α-amylase.

A serious problem in these days called "an age of gluttony" is that many people suffer from corpulence and an adult disease or geriatric disease as a consequence of corpulence so that dieting or control of food intake is an important means for health control. In the midst of this current, dietary fibers, which cannot be absorbed as food, are highlighted and utilized in various aspects. The effect of dietary fibers consists in the control of the absorption of harmful substance including carcinogenic ones and enhancement of the evacuating performance of the intestines rather than positive suppression of corpulence.

α-Amylase is a kind of digestive enzymes capable of hydrolyzing polysaccharides and contained in the saliva and pancreatic juice of human. Accordingly, inhibition of the activity of α-amylase would hopefully have an effect to prevent corpulence with adequate satisfaction of the appetite and exhibit a therapeutic effect for

diabetes. Several inhibitive agents against the activity of α-amylase have been developed with such an object although none of them is quite satisfactory in the activity with certain undesirable side effects in some of them.

Accordingly, it is eagerly to develop a novel inhibitive agent against activity of α-amylase which can be administered to patients without particular care to undesirable side effects against human body.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a novel inhibitive agent against activity of α-amylase as mentioned above. The inventor has continued extensive investigations of natural products to discover a substance capable of exhibiting the desired effect without the problems usually ensure in chemically synthesized compounds.

Thus, the inhibitive agent of the present invention against activity of α-amylase comprises tea polyphenol as the medicinally effective ingredient.

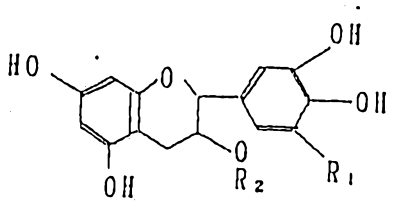
The polyphenol compound in tea as the effective ingredient in the inhibitive agent is selected from the group consisting of epigallocatechin gallate, epicatechin gallate, ~~epigallocatechin, epicatechin, (+) catechin~~ and the isomers thereof, free theaflavin, theaflavin monogallate A, theaflavin monogallate B and theaflavin digallate.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method of inhibiting the activity of α-amylase in a patient in need thereof which comprises administering an effective amount of a tea polyphenol.

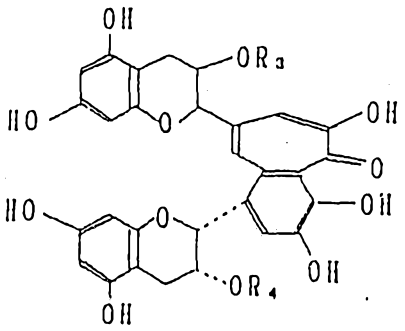
The tea polyphenol compounds as the principal

effective ingredients in the inventive inhibitive agent against activity of α-amylase include the tea catechin compounds represented by the general formula (I) given below and the theaflavin compounds represented by the general formula (II) given below:



, ... (I)

in which R_1 is a hydrogen atom or a hydroxy group and R_2 is a hydrogen atom or a 3,4,5-trihydroxy benzoyl group; and



, ... (II)

in which R_3 and R_4 are, each independently from the other, a hydrogen atom or a 3,4,5-trihydroxy benzoyl group.

Particular examples of the tea catechin compounds represented by the general formula (I) include: (-)epicatechin, which is a compound of the formula (I) with R_1 = H and R_2 = H; (-)epigallocatechin, which is a compound of the formula (I) with R_1 = OH and R_2 = H; (-)epicatechin gallate, which is a compound of the formula (I) with R_1 = H and R_2 = 3,4,5-trihydroxy benzoyl group; and (-)epigallocatechin gallate, which is a compound of the formula (I) with R_1 = OH and R_2 = 3,4,5-trihydroxy benzoyl group. Particular examples of the theaflavin compounds include: free theaflavin, which is a compound of the formula (II) with R_3 = H and R_4 = H; theaflavin monogallate A, which is a compound of the formula (II) with R_3 = 3,4,5-trihydroxy

benzoyl group and R_4 = H; theaflavin monogallate B, which is a compound of the formula (II) with R_3 = H and R_4 = 3,4,5-trihydroxy benzoyl group; and theaflavin digallate, which is a compound of the formula (II) with R_3 = 3,4,5-trihydroxy benzoyl group and R_4 = 3,4,5-trihydroxy benzoyl group.

The above described tea polyphenol compounds can be prepared from tea leaves as the starting material and a method for the preparation thereof and a typical example of the product composition are described, for example, in Japanese Patent Kokai 59-219384, 60-13780 and 61-130285 and elsewhere.

When the inventive inhibitive agent against activity of α-amylase is to be processed into a medicament form or an additive for food etc., the above described tea polyphenol as the effective ingredient as such is admixed with the base without or with dilution with water or alcohol. In this case, the concentration thereof in the digestive tract is preferably in the range from 0.1 μM to 5 mM or, more preferably, form 0.5 μM to 1 mM.

The above described inhibitive agent against activity of α-amylase comprises, as the effective ingredient, a natural product which is a drinkable taken in daily life in a considerably large volume so that it is absolutely free from the problem of undesirable side effects against human body not only when it is used as a medicine but also

when it is used as an additive of food. Moreover, the effectiveness thereof is so high that activity of α-amylase can be effectively inhibited by the addition thereof even in a very low concentration to provide a means for inhibiting activity of α-amylase.

In the following, examples are given to illustrate the invention in more detail.

Example 1

The enzyme used here was a product of α-amylase prepared from human saliva and supplied by Sigma Co.

A 150 μl of the enzyme solution (0.44 U/ml in a buffer solution) was added to 1230 μl of the sample solution and the mixture was incubated at 37°C for 10 minutes. Thereafter, the sample solution was admixed with 120 μl of a solution of soluble starch as the substrate so as to have a final concentration of the substrate of 2.0 mg/ml to effect the reaction at 37°C. A 200 μl of the solution was taken in every 3 minutes from the solution under proceeding reaction and the reducing sugar produced therein was determined by the measurement of the absorbance at a wavelength of 540 nm according to the method of Bernfeld described in Meth. Enzymol., volume 1, page 49 (1959) by P. Bernfeld. The value of the absorbance was converted by calculation into the amount of maltose from which the reaction velocity was calculated according to the

conventional procedure. The concentration of the solution for 50% inhibition of the activity of α-amylase was determined with each sample assuming that the activity of α-amylase was 100% when the reaction velocity was equal to that in the control in which the same volume of the buffer solution was added in place of the sample solution. The results are shown in Table 1 below.

Table 1

Sample	Concentration for 50% inhibition
Gallic acid	>> 1mM
Epicatechin	>> 1mM
Isomer of epicatechin	>> 1mM
Epicagallocatechin	>> 1mM
Isomer of epigallocatechin	>> 1mM
Epicatechin gallate	130 μM
Isomer of epicatechin gallate	20 μM
Epigallocatechin gallate	260 μM
Isomer of epigallocatechin gallate	55 μM
Free theaflavin	18 μM
Theaflavin monogallate A	1.0 μM
Theaflavin monogallate B	1.7 μM
Theaflavin digallate	0.6 μM

A conclusion could be derived from the above given ~~results~~ results that, among the catechin compounds shown in the

table, epicatechin, epigallocatechin and isomers thereof have almost no power for the inhibition of the activity of α-amylase but the other catechin compounds and theaflavin compounds have strong power for the inhibition of the activity of α-amylase.

Example 2

Each of the 12-weeks old male rats of the Wistar strain, divided into a test group and a control group, was fed a high-carbohydrate diet either with or without, respectively, of 1% by weight of Polyphenon 100 which was a crude mixture of catechin compounds in a proportion shown in Table 2 below.

Table 2

Catechin compound	Polyphenon 100	
	Content, %	Relative content, %
Gallocatechin	1.44	1.6
Epigallocatechin	17.57	19.3
Catechin	-	-
Epicatechin	5.81	6.4
Epigallocatechin gallate	53.90	59.1
Epicatechin gallate	12.51	13.7
Total	91.23	100

The formulation of the high-carbohydrate diet given to the control animals was as shown below in Table 3. In the diet given to the test animals, the formulation was modified by decreasing the amount of the starch powder to 70.0% and addition of 1.0% of Polyphenon 100 instead.

Table 3

Constituent	Content in high-carbohydrate diet (control), %	Content in the diet being added Polyphenon 100, %
Casein	22.0	22.0
Salt mix	4.0	4.0
Corn oil	2.0	2.0
Vitamin complex	1.0	1.0
Starch powder	71.0	70.0
Polyphenone 100	-	1.0
Total	100	100

After 7 days of raising in this manner, the feces discharged from each animal was collected for one day and weighed to examine the change in the amount thereof caused by the addition of Polyphenon 100 to the diet. The results were that the amount in the control animals was 1.01 g per day per animal while the amount in the test animals was 1.78 g per day per animal to support the conclusion that the addition of the catechin compounds to the diet was

effective to increase the amount of feces discharge. This result means that the catechin compounds act in a similar manner to dietary fibers in promoting the evacuating performance of the intestines by decreasing absorption of the carbohydrates as a consequence of the power to inhibit the activity of amylase.

Example 3

When the inventive inhibitive agent against the activity of α-amylase is administrated to human body, the dose to be taken orally is 0.1 to 10 g per day or, preferably, 2 to 5 g per day. The form of the medicament is not particularly limitative and it can be taken as such or in the form of a powder, tablet, capsule and the like, optionally, with admixture of an extending agent. When the inventive agent is used as an additive in food, it is added to various kinds of processed food and confectionery such as breads, noodles, cakes, biscuits, cookies and the like in an amount of 0.2 to 1.0% by weight.

Example 4

An animal test was conducted by using ICR mice as the test animals to examine the acute toxicity of the inventive inhibitive agent against the activity of α-amylase. The values of LD_{50} calculated according to the Van der Waerdrn method within the confidence limit were: 2412 mg/kg in the oral administration of the same crude mixture of catechin

compounds as used in Example 2; 55.2 mg/kg in the intraperitoneal administration of a crude mixture of theaflavin compounds of the composition shown in Table 5 below; and 150 mg/kg in the intraperitoneal administration of epigallocatechin gallate.

Table 5

Compound	Content, %, in the crude mixture of theaflavin compounds
Free theaflavin	10.0
Theaflavin monogallate A	22.3
Theaflavin monogallate B	19.5
Theaflavin digallate	32.5
(+) Catechin	0.3
(-) Epicatechin	1.8
(-) Epigallocatechin gallate	4.7
Isomer of (-) epigallocatechin gallate	1.0
(-) Epicatechin gallate	3.9
Others (isomers of theaflavin, etc.)	4.0

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of inhibiting the activity of α-amylase in a patient in need thereof which comprises administering an effective amount of a tea polyphenol.

2. A method as claimed in claim 1 wherein the tea polyphenol is selected from the group comprising epigallocatechin gallate, epicatechin gallate, and the isomers thereof, free theaflavin, theaflavin monogallate A, theaflavin monogallate B and theaflavin digallate.

3. A method as claimed in claim 1 or 2 wherein said tea polyphenol is administered orally.

4. A method as claimed in claim 3 wherein said tea polyphenol is administered in an amount of 0.1 to 10g per day.

5. A method as claimed in claim 3 wherein said tea polyphenol is administered in an amount of 2 to 5g per day.

6. A method as claimed in any one of claims 1 to 4 wherein said tea polyphenol is administered in the form of a powder, tablet or capsule which optionally contains a pharmaceutically acceptable carrier.

7. A method as claimed in any one of claims 1 to 5 wherein said tea polyphenol is administered in the form of a food additive.

8. A method as claimed in claim 7 wherein said tea polyphenol is present in the food in an amount of 0.2 to 1.0% by weight.

9. A method as claimed in claim 1 substantially as hereinbefore defined with reference to the examples.

DATED: 8 July 1992

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