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(54) **Title:** NOVEL STIGMASTEROL DERIVATIVE OR PHARMACEUTICALLY ACCEPTABLE SALT THEREOF, METHOD OF PRODUCING THE SAME, AND COMPOSITION CONTAINING THE SAME TO INHIBIT OBESITY OR TO PREVENT AND TREAT HYPERLIPIDEMIA

(57) **Abstract:** The present invention relates to a novel stigmasterol derivative or a pharmaceutically acceptable salt thereof, a method of producing the same, and a composition comprising the same for inhibiting obesity or for preventing and treating hyperlipidemia. The stigmasterol derivative according to the present invention is advantageous in that the stigmasterol derivative reduces the contents of triglyceride, total cholesterol, and LDL-cholesterol in blood, increases the content of HDL-cholesterol, and suppresses an increase in body weight. Accordingly, it is possible to use the stigmasterol derivative according to the present invention as medicines and health foods that are useful to suppress obesity or to prevent and treat hyperlipidemia.

# Description

# NOVEL STIGMASTEROL DERIVATIVE OR PHARMACEUTICALLY ACCEPTABLE SALT THEREOF, METHOD OF PRODUCING THE SAME, AND COMPOSITION CONTAINING THE SAME TO INHIBIT OBESITY OR TO PREVENT AND TREAT HYPERLIPIDEMIA

## Technical Field

[1] The present invention relates to a novel stigmasterol derivative or a pharmaceutically acceptable salt thereof, a method of producing the same, and a composition containing the same to inhibit obesity or to prevent and treat hyperlipidemia.

## Background Art

[2] In recent years, due to growth in the economy and changes in life style, dietary habits have significantly changed. In particular, excess body weight and obesity due to ingestion of high calorie food such as fastfood and insufficient exercise have considerably increased.

[3] The excess body weight has a body mass index (BMI, an index of obesity that is obtained by dividing weight by the square of height) in the range of 25 or more and less than 30. The obesity means that the body mass index is in the range of 30 or more.

[4] Two-thirds of Americans suffer from excess body weight or obesity. Excess body weight increases blood pressure and the amount of cholesterol, which causes various types of diseases in adults such as cardiac disorders, and obesity increases heart diseases, diabetes, arthritis, and some cancer.

[5] Excess body weight and obesity act as factors that increase the rate of occurrence of various types of diseases in adults such as arteriosclerosis, hypertension, hyperlipidemia or cardiac disorders in children or teenagers as well as adults.

[6] Obesity affects all age groups, and frequently occurs in the prime of life. It is known that obesity does not frequently occur in Asia due to their dietary habit of eating vegetables. However, currently, dietary habits and a residence environment are changing in Asia due to an influence of the Western civilization, thus increasing obesity.

[7] In addition, the modern people have reduced strength due to overworking, excessive drinking, stress, or the like, and it is known that about 30~40% of them are obese and about 10% of them are seriously obese.

[8] Obesity means that fat is excessively accumulated in the body and the ratio of fat to body weight is relatively high. Obesity is classified into simple obesity and secondary obesity (symptomatic obesity).

[9] Simple obesity may be caused by overeating, insufficient exercise, and a reduced basal metabolic rate. Most of the cases that are clinically diagnosed are simple obesity. If simple obesity continues over a long period of time, various types of disorders may occur.

[10] Secondary obesity is caused by certain basis diseases and examples thereof may include endocrine obesity, hypothalamus obesity, hereditary obesity, or obesity resulting from medicine.

[11] The main cause of obesity is due to the excessive growth of adipocytes, and the excessive growth of adipocytes is caused by the lack of hormone, which is called leptin that is secreted by the order of the brain. The lack of leptin hormone is caused by overeating, reduced metabolism in the living body due to aging, and animal cholesterol that is excessively accumulated in the body, with the exception of the case of the hereditary leptin hormone deficiency.

[12] Cholesterol includes small spherical lipoprotein particles in the blood. Lipoprotein is classified into various types. Lipoprotein that carries cholesterol from the liver to the other tissues is called low density lipoprotein (LDL), and lipoprotein that carries cholesterol from the other tissues to the liver is called high density lipoprotein (HDL). Particularly, HDL-cholesterol functions to clean the blood vessel to reduce arteriosclerosis. Thus, in the case when the content of HDL-cholesterol is low, the occurrence of arteriosclerosis is increased similar to the case when the content of LDL-cholesterol is high, which is considered to be unhealthy.

[13] Obesity incurs diseases such as hyperlipidemia, hypertension, cardiovascular disease, pseudotumor cerebri, sleep apnea, cancer, pulmonary hypertension, cholecystitis, and osteoarthritis, or reduces movement. Furthermore, obesity may be a complication resulting from various types of diseases.

[14] Generally, obesity occurs when ingestion energy continues while being more than consumption energy. Some obese patients make an effort to reduce body weight and cholesterol by diet therapy and exercise therapy for the purpose of treating obesity. Additionally, patients are subjected to behavioral treatment, psychotherapy, drug treatment, or bariatric surgery (a surgical operation such as gastric banding).

[15] Diet therapy is a method of controlling the total energy intake. However, diet therapy is problematic in that since a metabolic rate is reduced during rest, it is difficult to

ensure a sufficient weight reduction. In addition, even if the sufficient weight reduction is ensured, weight other than fat weight is reduced, such that physical and mental pains such as malnutrition disorder, hunger, or stress occur.

[16] In the case of exercise therapy, consumption energy is increased, the metabolic rate is improved during rest, resistance to insulin is avoided, and fat is reduced from the body. However, since it is necessary to do an aerobic exercise for 20 min per time and repeat the exercise three times or more a week, it is difficult to consistently maintain an exercise regimen.

[17] Behavioral treatment or psychotherapy is used as an auxiliary means of diet therapy or exercise therapy, and not considered as a main treatment to obtain the desired effect.

[18] In the case where the above-mentioned treatments are not useful or urgent treatment needs to be used because of serious obesity, drug treatment or bariatric surgery is used. However, bariatric surgery imposes a very heavy burden on a patient. Examples of medicine that is used to treat obesity by using the drug treatment include appetite depressants, digestion and absorption inhibitors, obesity inhibitors, or metabolism promotion agents. Furthermore, the medicine may incur a side effect such as drug dependence. Since a patient may have a tolerance to medicine over a short period of time, it is difficult to perform the treatment over a long period of time.

[19] Additionally, in the case where exercise therapy is used, if an obese person does a high-impact exercise, the possibility of damage to underlying joints is increased. Therefore, it is preferable that the obese person do a low-impact exercise such as swimming. In this case, preferably, medicine that has a low side effect and activation of energy metabolism may be used while the patient does a low-impact exercise such as swimming to increase muscle mass and promote consumption of energy due to an increase in basal metabolism, thereby reducing the possibility of obesity.

[20] Meanwhile, stigmasterol is phytosterol that is contained in soybean milk and has a structure similar to that of animal cholesterol. However, stigmasterol is absorbed in the guts while competing with animal cholesterol to reduce the concentration of cholesterol in the body and suppress the excessive growth of adipocytes. In the case of stigmasterol deficiency, calcium phosphate is accumulated in the joints or muscles and muscular atrophy occurs.

[21] Glucosamine is one of natural amino sugars that constitute the joints and the cartilages and its molecular formula is $C_6H_{13}NO_6$. Glucosamine has 6 carbon atoms, and glucosamine and galactosamine are generically known as hexosamine. Glucosamine is a strong basic material that has colorless needle crystals, and is

decomposed at 110°C and dissolved in water. Natural glucosamine is present in a chitin form or a polysaccharide form such as mucopolysaccharides constituting the cell walls of bacteria or the cartilages or skins of animals. A large amount of proteins that are bonded to glucosamine are contained in blood or mucus of humans, and glycolipid that is bonded to glucosamine is present in the cell membrane of a red blood cell. Furthermore, chitin may be decomposed using a hydrochloric acid to produce glucosamine. During biosynthesis of glucosamine, glucosamine prevents acetate from being provided into the cell to suppress formation of acetyl CoA. As a result, the synthesis of fats and cholesterol is obstructed. Since glucosamine is known as a material that helps make the joint and the cartilage strong, it is extensively used in medicines for degenerative arthritis, cosmetic compositions, foods, and the like.

[22] Currently, the increase in obesity has become a social concern, and there is a need to develop goods that are capable of significantly reducing the amount of cholesterol and preventing adipocytes from growing in the body and efficiently reducing the weight unlike known stigmasterol or glucosamine.

## Disclosure of Invention

## Technical Problem

[23] With respect to this, the present inventors have conducted studies on a compound capable of reducing the amount of cholesterol and preventing adipocytes from growing in the body, which has resulted in the finding of a novel stigmasterol derivative produced by the present inventors that reduces the contents of triglyceride, total cholesterol, and LDL-cholesterol in the blood, increases the content of HDL-cholesterol, and prevents body weight from being increased, thereby accomplishing the present invention.

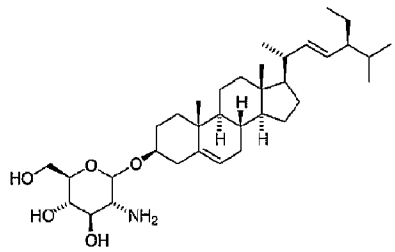
## Technical Solution

[24] The present invention provides a novel stigmasterol derivative or a pharmaceutically acceptable salt thereof, a method of producing the same, and a composition containing the same to inhibit obesity or to prevent and treat hyperlipidemia.

## Best Mode for Carrying Out the Invention

[25] According to an embodiment of the present invention, a novel stigmasterol derivative represented by the following Formula 1 or a pharmaceutically acceptable salt thereof is provided.

[26] [Formula 1]

[27] 

[28] The compound of the present invention may be used in the form of a pharmaceutically acceptable salt and a solvate according to the conventional method in the related art.

[29] As the pharmaceutically acceptable salt, acid addition salts produced with free acids are preferred. The acid addition salts are produced by the conventional method, for example, a method comprising the steps of dissolving a compound in an excessive amount of acid aqueous solution, and precipitating the salt using water-miscible organic solvents such as methanol, ethanol, acetone or acetonitrile. Acid or alcohol (for example, glycol monomethyl ether) in the same molar amount of compound and water is heated, and the mixture is dried by evaporation or the precipitated salt can be suction-filtered. At this time, as the free acids, organic acids and inorganic acids may be used. Examples of the inorganic acids include hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, and tartaric acid, and examples of the organic acids include methanesulfonic acid, p-toluenesulfonic acid, acetic acid, trifluoroacetic acid, citric acid, maleic acid, succinic acid, oxalic acid, benzoic acid, tartaric acid, fumaric acid, mandelic acid, propionic acid, lactic acid, glycollic acid, gluconic acid, galacturonic acid, glutamic acid, glutaric acid, glucuronic acid, aspartic acid, ascorbic acid, vanillic acid, and hydroiodic acid, but are not limited thereto.

[30] Further, a pharmaceutically acceptable metal salt can be produced using a base. An alkali metal salt and alkaline earth metal salt can be obtained by a method, in which a compound is dissolved in an excessive amount of alkali metal hydroxide or alkaline earth metal hydroxide solution, filtered the undissolved salt, and then the filtrate is evaporated and dried. In respects to metal salts, it is preferable that sodium, potassium, or calcium salt is pharmaceutically preferable, and the corresponding silver salt is obtained by reacting alkali metal salt or alkaline earth metal salt with a suitable silver salt (e.g. silver nitrate).

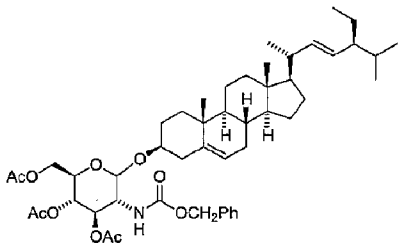
[31] A pharmaceutically acceptable salt of the compound represented by Formula 1 includes salts of acidic or basic groups, which can be present in the compound of Formula 1 unless otherwise specified. For example, the pharmaceutically acceptable

salt includes sodium salt, calcium salt, potassium salt of a hydroxy group, and other pharmaceutically acceptable salt of an amino group includes hydrochloride, hydrobromide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, acetate, succinate, citrate, tartrate, lactate, mandelate, methanesulfonate (mesylate), and p-toluenesulfonate (tosylate). Further, the salts can be produced by a known method for producing a salt or a production process in the related art.

[32] In another embodiment of the present invention, an intermediate compound of the stigmasterol derivative represented by the following Formula 3 or 4 or a pharmaceutically acceptable salt thereof is provided.

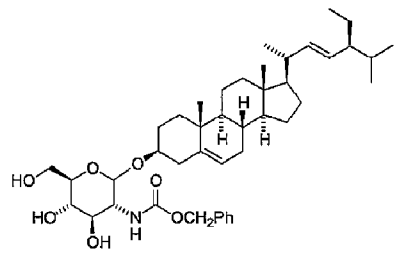
[33] [Formula 3]

[34]




[35] [Formula 4]

[36]




[37]

[38] According to another embodiment of the present invention, a method of producing the stigmasterol derivative represented by the above Formula 1 is provided.

[39] As shown in the following Reaction Scheme 1, the method of producing the stigmasterol derivative of the present invention comprises the steps of:

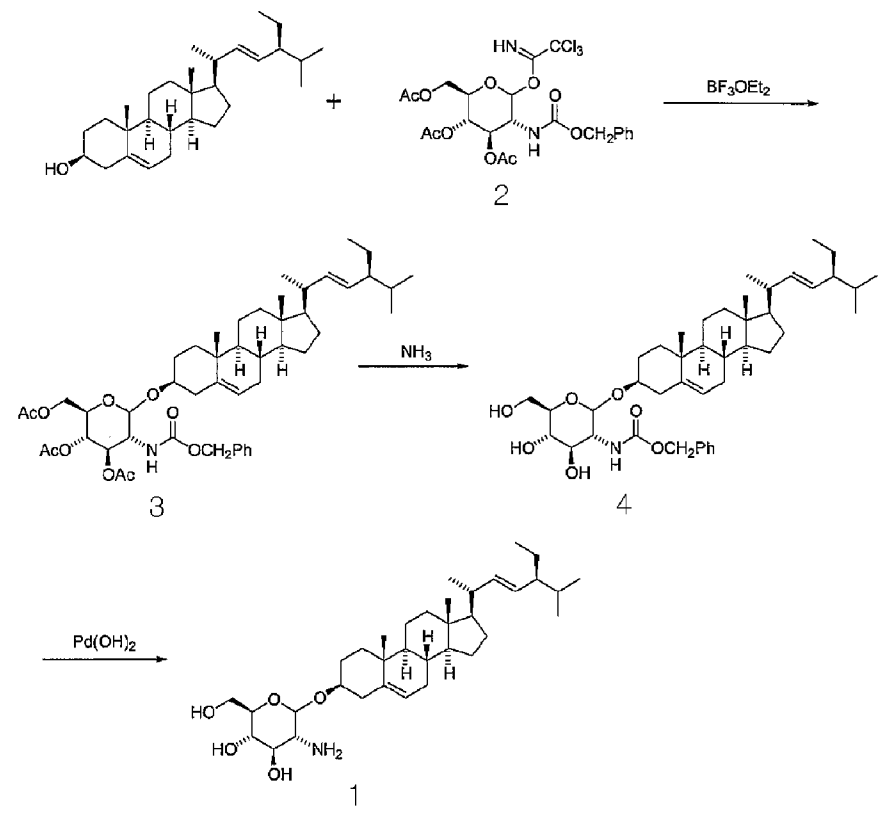
[40] 1) producing the compound of Formula 3 by reacting stigmasterol and the compound of Formula 2,

[41] 2) producing the compound of Formula 4 by reacting the compound of Formula 3 produced in step 1) and an ammonia aqueous solution, and

[42] 3) producing the compound of Formula 1 by reacting the compound of Formula 4 produced in step 2) and palladium hydroxide [$Pd(OH)_2$].

[43] [Reaction Scheme 1]

[44]




[45] In Reaction Scheme 1, the compound of Formula 2 as a starting material can be produced by the method described in Chemical. Reviews 1993, Vol. 93, No. 4, p. 1511 or the like or the similar method thereto, and used.

[46] In step 1), stigmasterol and the compound of Formula 2 are dissolved in an organic solvent, and then $BF_3OEt_2$ was added thereto. The mixture is subjected to reaction at normal temperature for 10~20 hours to produce the compound of Formula 3.

[47] The organic solvent is preferably selected from the group consisting of dimethyl ether, tetrahydrofuran, methylene chloride, methanol, cyclohexene, acetonitrile, dimethylformamide, and dimethyl sulfoxide, but is not limited thereto.

[48] In step 2), the compound of Formula 3 produced in step 1) is dissolved in an organic solvent, and reacted with the ammonia aqueous solution in a water bath at 30~50°C for 10~20 hours to produce the compound of Formula 4.

[49] The organic solvent can be selected from the organic solvents used in step 1). The base is preferably selected from the group consisting of sodium hydride, potassium hydride, sodium hydroxide, potassium hydroxide, sodium carbonate, potassium carbonate, cesium carbonate, sodium bicarbonate, potassium bicarbonate, tri-ethylamine, pyridine, DBU, and ammonium hydroxide, but is not limited thereto.

[50] In step 3), the compound of Formula 4 produced in step 2) is dissolved in an organic solvent and distilled water, and reacted with palladium hydroxide[$Pd(OH)_2$] to produce the compound of Formula 1. The organic solvent used herein can be selected from the organic solvents used in step 1).

[51]

[52] According to still another embodiment of the present invention, a composition containing the stigmasterol derivative represented by Formula 1 or the pharmaceutically acceptable salt thereof to inhibit obesity or to prevent and treat hyperlipidemia is provided.

[53] The stigmasterol derivative according to the present invention has the excellent effects of decreasing blood levels of triglyceride, total cholesterol, and LDL cholesterol, increasing blood level of HDL-cholesterol, and inhibiting body weight gain.

[54] Further, toxicity test was performed by orally administering the stigmasterol derivative according to the invention to a mouse, resulting in a Lethal Dose 50 ($LD_{50}$) after oral administration of 3000 ㎎/㎏ or more, which indicates that the stigmasterol derivative is a safe substance.

[55] Accordingly, the stigmasterol derivative according to the present invention can be used as a medicine and as health food useful for inhibiting obesity or for preventing and treating hyperlipidemia.

[56] The composition of the invention may contain at least one kind of active ingredient known in the art, which has the effect of inhibiting obesity or preventing or treating hyperlipidemia, in addition to the compound of Formula 1.

[57] For administration, the composition of the invention can include at least one pharmaceutically acceptable carrier, in addition to the active ingredients as described above. Examples of the pharmaceutically acceptable carrier include saline solution, sterile water, Ringer's solution, buffered saline solution, dextrose solution, maltodextrin solution, glycerol, ethanol and a mixture of one or more thereof. If necessary, the composition may also contain other conventional additives such as antioxidants, buffers, and bacteriostatic agents. Moreover, the composition may additionally contain diluents, dispersants, surfactants, binders, and lubricants in order to formulate it into injectable formulations such as aqueous solution, suspension, and emulsion, pills, capsules, granules, and tablets. Furthermore, the composition may preferably be formulated depending on particular diseases and its components, using the method described in Remington's Pharmaceutical Science (latest edition), Mack Publishing

Company, Easton PA, which is a suitable method in the relevant field of art.

[58] The composition of the invention may be administered orally or parenterally (for example, intravein, subcutaneous, intraperitoneal, or topical application). The dosage of the composition of the invention can vary depending on various factors, including the patient's weight, age, sex, health condition, and diet, and administration time, administration route, secretion rate, disease severity, etc. The compound of Formula 1 is administered at a daily dosage of about 5~30 mg/kg, preferably 10~25 mg/kg one time or several times.

[59] The composition of the invention may be used alone or in combination with surgical operations, hormone therapies, chemical therapies, and other methods using biological reaction regulators, in order to inhibit obesity or to prevent or treat hyperlipidemia.

[60] The composition of the present invention can be added to a health food for the purpose of improving the diseases caused by obesity or hyperlipidemia. In the case of using the compound of Formula 1 of the invention as a food additive, the compound of Formula 1 can be added as it is or with other foods or food ingredients, and suitably used according to the conventional method. The mixed amount of active ingredient can be suitably determined depending on its purpose (prevention, health, or treatment). Generally, during the production of food or beverage, the compound of Formula 1 of the invention is added 15% by weight or less, preferably 10% by weight or less, based on a raw material. However, in the case of taking over a long period of time for the purpose of health and hygiene or for the purpose of controlling health, the amount may be less than the above range. Since there is no problem in safety, the active ingredient may be used in an amount more than the above range.

[61] The kind of food is not particularly limited. Examples of the food, to which the substance can be added, include meat, sausage, bread, chocolate, candy, snack, cookie, pizza, instant noodle, other noodles, chewing gum, dairy products including icecream, various kinds of soup, beverage, tea, drink, alcoholic beverages and vitamin complex, and includes all kinds of typical health foods.

[62] The health beverage composition of the present invention may contain various flavors, natural carbohydrates or the like as an additional ingredient, similarly to a conventional beverage. Examples of the natural carbohydrates include monosaccharides such as glucose, fructose, disaccharides such as maltose, sucrose, polysaccharides such as dextrin and cyclodextrin, sugaralcohols such as xylitol, sorbitol, erythritol. Examples of the sweeteners include natural sweeteners such as thaumatin and stevioside, and artificial sweeteners such as saccharin and aspartame. The content of

the natural carbohydrate is generally about 0.01~0.04 g, preferably about 0.02~0.03 g, based on 100 ml of the composition of the present invention.

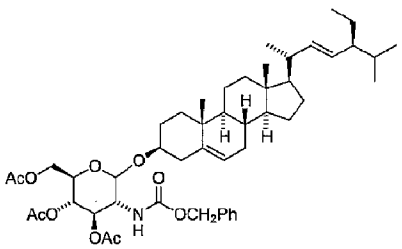
[63] The composition of the invention may contain various nutrients, vitamins, minerals, flavoring agent, coloring agent, pectic acid and a salt thereof, alginic acid and a salt thereof, organic acid, protective colloid thickener, pH control agent, stabilizer, preservative, glycerin, alcohol, carbonating agent used in carbonated beverages or the like, in addition to the above-mentioned. Further, the composition of the invention may contain fruit fleshes for production of natural fruit juice, fruit juice beverage, and vegetable beverage. The above ingredients can be used alone or in combination therewith. Generally, the content of the additives is, but not considered significant, 0.01~0.1 parts by weight, based on 100 parts by weight of the composition of the present invention.

## Mode for the Invention

[64] Hereinafter, the preferred Examples are provided for better understanding. However, these Examples are for the illustrative purpose only, and the invention is not intended to be limited by these Examples.

[65] **EXAMPLE 1 : Production of stigmasterol derivative**

[66] **1. Production of acetic acid 4-acetoxy-2-acetoxymethyl-5-benzyloxycarbonylamino-6-[17-(4-ethyl-1,5-dimethyl-2-hexenyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy]-tetrahydro-pyran-3-yl ester (3)**

[67] 


[68] Acetic acid 4-acetoxy-2-acetoxymethyl-5-benzyloxycarbonylamino-6-(2,2,2-trichloroacetimidoyloxy)-tetrahydro-pyran-3-yl ester (10 g, 0.0171 mol) and stigmasterol (7.0 g, 0.0171 mol) were dissolved in 250 ml of dimethyl ether, 0.69 ml of $BF_3OEt_2$ was added thereto, and the mixture was stirred at normal temperature for 16 hours. After the reaction was completed, the reaction solvent was removed to obtain a concentrate. After the concentrate was dissolved in methylene chloride, separation was performed by means of silica gel column chromatography using a solvent mixture of ethyl acetate

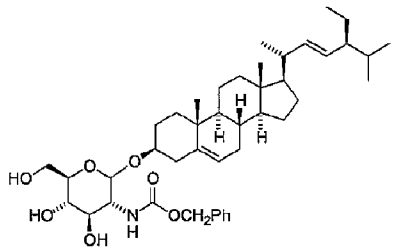
: hexane (1:3) to obtain the compound (3).

[69] Yield : 21%,

[70] Melting point : 165~167℃,

[71] $^1$H-NMR(500MHz, $CDCl_3$) : 0.68-2.28(43H, m), 1.97(3H,s), 2.01(3H,s), 2.07(3H,s), 3.47(2H,m), 3.61(1H,m), 4.10(1H,d), 4.25(1H,m), 4.80(1H, m), 5.02(2H,t), 5.11(3H,m), 5.30(1H,s), 5.35(1H,m), 7.33(5H,s)

[72] **2. Production of {2-[17-(4-ethyl-1,5-dimethyl-2-hexenyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17,-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxyl]-4,5-dihydro-6-hydroxymethyl-tetrahydro-pyran-3-yl}-carbamic acid benzyl ester (4)**

[73] 

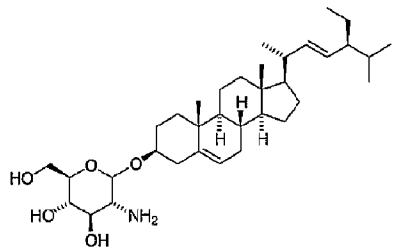
[74] Acetic acid 4-acetoxy-2-acetoxymethyl-5-benzyloxycarbonylamino-6-[17-(4-methyl-1,5-dimethyl-2-hexenyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy]-tetrahydro-pyran-3-yl ester (5.0 g, 0.006 mol) that was produced in the above 1 was dissolved in 30 ㎖ of methylene chloride, 80 ㎖ of methanol was added, 25 ㎖ of ammonia aqueous solution was added, and the resulting solution was stirred in a water bath at 40℃ for 16 hours while the stopper was put on the reactor. After the reaction, produced solids were filtered, washed with water, and dried to obtain the compound (4).

[75] Yield : 85%,

[76] Melting point : 193~195℃,

[77] $^1$H-NMR(500MHz, $CDCl_3$) : 0.68-2.26(43H, m), 3.29(2H,m), 3.42(1H, m), 3.5-3.8(3H,m), 4.58(2H,m), 5.10(3H,s), 5.28(2H,s), 6.10(1H,m), 7.30(5H,s)

[78] **3. Production of 5-amino-6-[17-(4-ethyl-1,5-dimethyl-hexenyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxy]-2-hydromethyl-tetrahydro-pyran-3,4-diol (1)**

[79] 

[80] 220 ㎖ of methanol, 110 ㎖ of cyclohexene, and 20 ㎖ of distilled water were sequentially added to {2-[17-(4-ethyl-1,5-dimethyl -hexenyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxyl]-4,5-dihydro-6-hydroxymethyl-tetrahydro-pyran-3-yl}-carbamic acid benzyl ester (2.0 g, 0.0028 mol) that was produced in the above 2, 5.0 g of palladium hydroxide [$Pd(OH)_2$] was added, and reflux was performed for 2 hours while stirring was carried out. After the reaction was completed, palladium was removed by using cellite and the filtered solution was concentrated. The concentrated solution was dissolved in a developing solvent (methylene chloride:methanol = 8:1) and separation was performed by using silica gel column chromatography to obtain the compound (1).

[81] Yield : 55%,

[82] Melting point : 255℃,

[83] $^1$H-NMR(500MHz, $CDCl_3$) : 0.68-2.17(43H, m), 2.42(2H,m), 3.11 (4H,m), 3.35(2H,m), 3.69(1H,m), 4.26(2H,m), 4.48(1H,t), 4.96(1H,s), 5.08(1H, m), 5.17(1H,m), 5.37(1H,s)

[84]

[85] **EXPERIMENTAL EXAMPLE 1 : Anti-obesity, anti-lipid and anti-cholesterol test**

[86] In order to evaluate anti-obesity, anti-lipid and anti-cholesterol abilities of the compound of Formula 1 according to the present invention, the following test was performed.

[87] 30 three-week-old SD male rats that were obtained from Central Lab. Animal, Inc. were used as subject animals. In respects to a breeding condition, the temperature was 23±3℃, the relative humidity was 55±10%, the number of ventilation was 10~20 ventilation/time, the lighting time of the fluorescent lamp was 12 hours/day (the switch was turned on at 8 a.m. and the switch was turned off at 8 p.m.), and the intensity of illumination was 150~300 Lux. The subject animals were divided into five groups, and the six rats were bred in each group in the polycarbonate breeding box (size : 220 W ×

360 L × 200 H ㎜).

[88] After the subject animals became adapted to new environment for 1 week, the rats were allowed to freely ingest AIN-76A high fat diet (Dytes Inc, USA) as feed for 4 weeks. Subterranean water that was acceptable to drink was sterilized by using a micro drum filter and a UV sterilizer and the rats were allowed to freely drink the sterilized water by using the sterilized water bottle.

[89] While the same feed was given to the rats that became fat for 6 weeks, distilled water was orally administered to the first group (negative control group), 400 mg/kg of the compound 1 that was produced in Example 1 was orally administered to the second group (test group 1), 800 mg/kg of the compound 1 that was produced in Example 1 was orally administered to the third group (test group 2), 800 mg/kg of stigmasterol that was the control medicine was orally administered to the fourth group (positive control group 1), and 800 mg/kg of glucosamine that was the control medicine was orally administered to the fifth group (positive control group 2).

[90] Before the test was finished, the rats that fasted for 16 hours were anesthetized using dimethyl ether and had their blood taken from the abdominal venae cavae. After the blood was centrifuged, serum was obtained. The contents of triglyceride, total cholesterol, and HDL-cholesterol in the serum were measured by using a quantitative kit reagent (ASAN Pharmaceutical Co.) according to an enzymatic colorimetric method, and the content of LDL-cholesterol was calculated by using the following Friedewald equation.

[91]

[92] LDL-cholesterol = [Total cholesterol - HDL-cholesterol - (triglyceride / 5)]

[93]

[94] The rats were isolated from each other, and the dietary intake and the weight were measured twice per week during the test.

[95] The content of triglyceride in the blood is described in Table 1, the content of total cholesterol in the blood is described in Table 2, the content of HDL-cholesterol in the blood is described in Table 3, and the content of LDL-cholesterol in the blood is described in Table 4. In addition, a change in body weight is described in Table 5.

[96] Table 1

[Table 1]

[Table ]

Content of triglyceride in blood

| | 6 weeks after sample was administered(㎎/㎗) | Decrease ratio (%) |
|---|---|---|
| Negative control group (distilled water) | 80.19±9.23 | - |
| Test group 1 (400 mg/kg) | 55.71±2.12 | -30.52 |
| Test group 2 (800 mg/kg) | 53.63±1.88 | -33.12 |
| Positive control group 1 (stigmasterol) | 74.29±8.26 | -7.30 |
| Positive control group 2 (glucosamine) | 71.54±6.53 | -10.70 |

[97] Table 2

[Table 2]

[Table ]

Content of total cholesterol in blood

| | 6 weeks after sample was administered (㎎/㎗) | Decrease ratio (%) |
|---|---|---|
| Negative control group (distilled water) | 82.09±5.45 | - |
| Test group 1 (400 mg/kg) | 73.27±12.65 | -10.70 |
| Test group 2 (800 mg/kg) | 71.75±13.72 | -12.59 |
| Positive control group 1 (stigmasterol) | 78.05±15.42 | -4.90 |
| Positive control group 2 (glucosamine) | 80.48±14.73 | -1.90 |

[98] Table 3

[Table 3]

[Table ]

Content of HDL-cholesterol in blood

| | 6 weeks after sample was administered (㎎/㎗) | Increase ratio (%) |
|---|---|---|
| Negative control group (distilled water) | 24.84±2.33 | - |
| Test group 1 (400 mg/kg) | 25.12±1.92 | +1.11 |
| Test group 2 (800 mg/kg) | 25.43±2.12 | +2.37 |
| Positive control group 1 (stigmasterol) | 24.53±3.12 | - |
| Positive control group 2 (glucosamine) | 24.47±1.86 | - |

[99] Table 4

[Table 4]

[Table ]

Content of LDL-cholesterol in blood

| | 6 weeks after sample was administered (㎎/㎗) | Decrease ratio (%) |
|---|---|---|
| Negative control group (distilled water) | 42.21±8.66 | - |
| Test group 1 (400 mg/kg) | 37.01±7.21 | -10.19 |
| Test group 2 (800 mg/kg) | 35.59±5.71 | -13.64 |
| Positive control group 1 (stigmasterol) | 38.66±6.34 | -6.20 |
| Positive control group 2 (glucosamine) | 41.70±7.26 | -1.19 |

[100]

[101] As shown in Tables 1 to 4, it could be seen that in the case where the compound according to the present invention was used, the content of triglyceride in the blood was reduced by 30~34%, the content of total cholesterol in the blood was reduced by

10~13%, the content of LDL-cholesterol in the blood was reduced by 10~14%, and the content of HDL-cholesterol in the blood was increased by 1~3%, as the results of biochemical test of blood in comparison with the high fat diet group (negative control group). Thus, it can be seen that the compound according to the present invention significantly improves the blood lipoprotein.

[102] However, in the case of stigmasterol and glucosamine that were the positive control medicines, the contents of triglyceride in the blood were reduced by 7.30% and 10.70%, the contents of total cholesterol in the blood were reduced by 4.90% and 1.90%, the contents of LDL-cholesterol in the blood were reduced by 6.20% and 1.19%, and the contents of HDL-cholesterol in the blood were insignificantly increased in comparison with the high fat diet group (negative control group). Thus, it can be seen that stigmasterol and glucosamine insignificantly improve the blood lipoprotein.

[103] Table 5

[Table 5]

[Table ]

Change in body weight

| | Before sample was administered (g) | 6 weeks after sample was administered (g) | Decrease ratio in body weight (%) |
|---|---|---|---|
| Negative control group (distilled water) | 223.8±6.3 | 386.0±13.1 | - |
| Test group 1(400 mg/kg) | 221.0±7.1 | 370.5±14.9 | -7.8 |
| Test group 2(800 mg/kg) | 223.3±6.9 | 365.4±15.5 | -12.4 |
| Positive control group 1 (stigmasterol) | 223.4±8.1 | 383.6±16.6 | -1.2 |
| Positive control group 2 (glucosamine) | 222.6±5.6 | 379.3±17.9 | -3.4 |

[104]

[105] As shown in Table 5, the compound according to the present invention reduces the weight by 7.8 to 12.4% in comparison with the high fat diet group (negative control group). In the case of stigmasterol and glucosamine that were the positive control medicines, the weights were reduced by 1.2% and 3.4% in comparison with the high

fat diet group (negative control group).

[106]

[107] **EXPERIMENTAL EXAMPLE 2 : Acute toxicity test**

[108] In order to evaluate acute toxicity of the stigmasterol derivative according to the present invention, the acute toxicity test was performed by using the Richfield-Wilcoxon method.

[109] Six-week-old ICR mice were used as test animals. The mice were allowed to freely ingest solid feed and water at the temperature of 23±1℃ and the humidity of 60±5% before the test was performed. The test animals were divided into groups, and six mice were included in each group. The compound 1 that was produced in Example 1 was suspended in a 0.5% methyl cellulose solution and then orally administered in the amount of 1 g/kg/15 ㎖ once. After the test material was administered, mice were observed for 14 days in views of appearance and death. The dead mice were subjected to an autopsy and observed in terms of grosslesions. The $LD_{50}$ value was obtained by using the Richfield-Wilcoxon method.

[110] From the test results, it could be seen that there was no clinical symptom in the mice to which the test material was administered, and no dead mice were found.

[111] Therefore, the compound of the present invention does not incur a change in toxicity until the amount is increased to 3000 ㎎/㎏ in respects to all the mice. Additionally, the compound is evaluated as a safe substance having the oral administration lethal dose 50 ($LD_{50}$) of 3000 ㎎/㎏ or more.

[112]

[113] Preparation Examples of the compositions of the present invention are described below.

[114] **PREPARATION EXAMPLE 1 : Production of pharmaceutical preparation**

[115] 1. Production of powder

[116] Compound of Formula 1 2 g

[117] Lactose 1 g

[118] The above-mentioned components were mixed with each other and then packed by using an airtight sheet to produce powder.

[119] 2. Production of tablet

[120] Compound of Formula 1 100 ㎎

[121] Corn starch 100 ㎎

[122] Lactose 100 ㎎

[123] Magnesium stearate 2 mg

[124] The above-mentioned components were mixed with each other and then tableted by using a typical production process to produce tablet.

[125] 3. Production of capsule preparation

[126] Compound of Formula 1 100 mg

[127] Corn starch 100 mg

[128] Lactose 100 mg

[129] Magnesium stearate 2 mg

[130] The above-mentioned components were mixed with each other and then packed in a gelatin capsule by using a typical production process of capsule preparation to produce capsule preparation.

[131]

[132] **PREPARATION EXAMPLE 2 : Production of foods**

[133] Foods that contain the compound of Formula 1 according to the present invention were produced by using the following methods.

[134] 1. Production of seasonings

[135] 20~95 % by weight of compound of Formula 1 was used to produce seasonings that were healthy.

[136] 2. Production of tomato ketchup and sauce

[137] 0.2~1.0 % by weight of compound of Formula 1 was added to tomato ketchup or sauce to produce tomato ketchup or sauce that was healthy.

[138] 3. Production of wheat flour foods

[139] 0.5~5.0 % by weight of compound of Formula 1 was added to wheat flour and the resulting mixture was used to produce bread, cake, cookies, crackers, and noodles. Thereby, foods that were healthy were obtained.

[140] 4. Production of soup and gravy

[141] 0.1~5.0 % by weight of compound of Formula 1 was added to soup and gravy to produce processed meat, and soup and gravy for noodles that were healthy.

[142] 5. Production of ground beef

[143] 10 % by weight of compound of Formula 1 was added to ground beef to produce ground beef that was healthy.

[144] 6. Production of dairy products

[145] 5~10 % by weight of compound of Formula 1 was added to milk and dairy products such as butter and ice cream were produced by using the milk.

[146]

[147] **PREPARATION EXAMPLE 3 : Production of beverages**

[148] 1. Production of carbonated beverages

[149] Additives such as 5~10% of sugar, 0.05~0.3% of citric acid, 0.005~0.02% of caramel, and 0.1~1% of vitamin C were mixed with each other and 79~94% of purified water was added thereto to produce a syrup. The syrup was sterilized at 85~98℃ for 20~180 sec and then mixed with cold water at a ratio of 1:4. 0.5~0.82% of carbon dioxide was added to the resulting mixture to produce a carbonated beverage that contained the compound of Formula 1 according to the present invention.

[150] 2. Production of beverages that were healthy

[151] Side materials such as fructose liquid (0.5%), oligosaccharide (2%), sugar (2%), common salt (0.5%), and water (75%) and the compound of Formula 1 were homogeneously mixed with each other and instantaneously sterilized. The sterilized mixture was packed by using small-sized vessels such as glass bottles and pet bottles to produce beverages that were healthy.

[152] 3. Production of vegetable juice

[153] 5 g of compound of Formula 1 was added to 1,000 ㎖ of tomato or carrot juice to produce vegetable juice that was healthy.

[154] 4. Production of fruit juice

[155] 1 g of compound of Formula 1 was added to 1,000 ㎖ of apple or grape juice to produce fruit juice that was healthy.

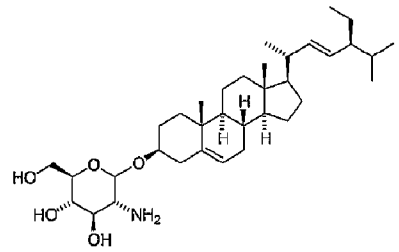
## Industrial Applicability

[156] The stigmasterol derivative according to the present invention is advantageous in that the stigmasterol derivative reduces the contents of triglyceride, total cholesterol, and LDL-cholesterol in blood, increases the content of HDL-cholesterol, and suppresses an increase in body weight. Accordingly, it is possible to use the stigmasterol derivative according to the present invention as medicines and health foods that are useful to suppress obesity or to prevent and treat hyperlipidemia.

# Claims

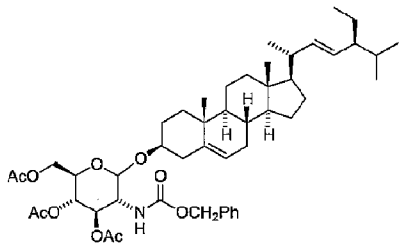
[1] A stigmasterol derivative represented by Formula 1 or a pharmaceutically acceptable salt thereof.

[Formula 1]



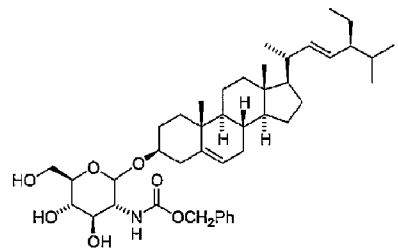
[2] An intermediate compound of a stigmasterol derivative represented by Formula 3 or a pharmaceutically acceptable salt thereof.

[Formula 3]



[3] An intermediate compound of a stigmasterol derivative represented by Formula 4 or a pharmaceutically acceptable salt thereof.

[Formula 4]



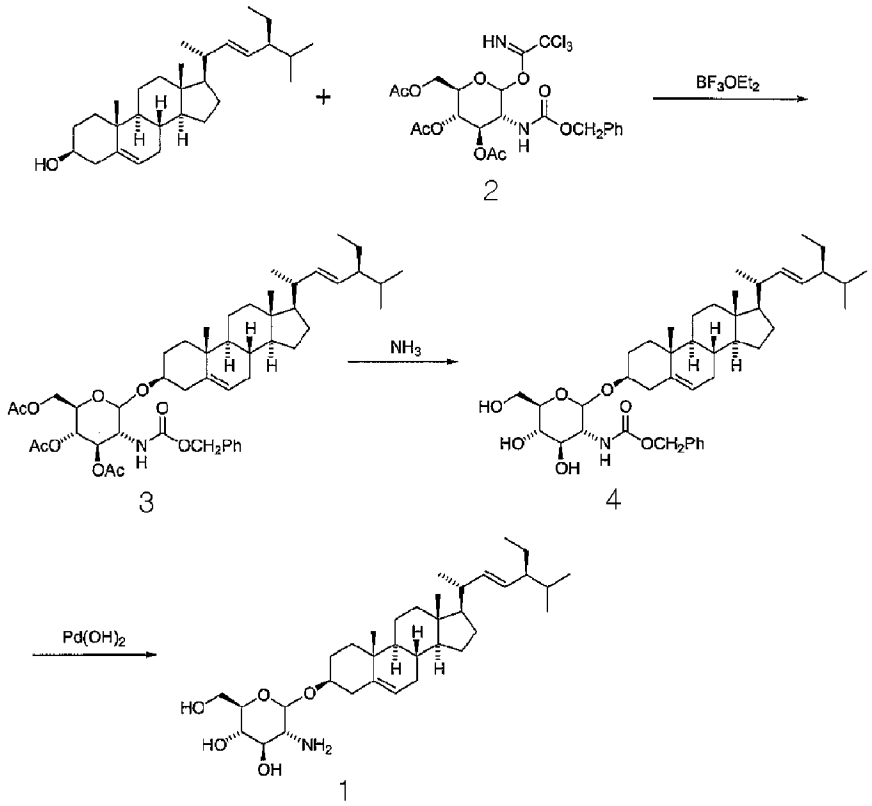
[4] A method of producing the compound of claim 1, which is represented by Reaction Scheme 1, the method comprising the steps of:

1) producing the compound of Formula 3 by reacting stigmasterol and the compound of Formula 2;

2) producing the compound of Formula 4 by reacting the compound of Formula 3 produced in the step 1) and an ammonia aqueous solution; and

3) producing the compound of Formula 1 by reacting the compound of Formula 4 produced in the step 2) and palladium hydroxide [$Pd(OH)_2$].

[Reaction Scheme 1]




[5] A pharmaceutical composition containing the compound of claim 1 to suppress obesity or to prevent and treat hyperlipidemia.

[6] A food composition that contains the compound of claim 1 and is useful to suppress obesity or to prevent hyperlipidemia.

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/KR2007/004395**

**A. CLASSIFICATION OF SUBJECT MATTER**

*C07J 9/00(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS(KIPO internal), esp@cenet, Delphion, ScienceDirect, STN "stigmasterol, cholesterol, and derivative"

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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| A | WO 2000/052029 A1 (EUGENE SCIENCE INC.) 8 September 2000<br>See Page 4 line 25-Page 19 line 5. | 1-6 |
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| A | WO 2005/005453 A2 (FORBES MEDI-TECH INC.) 20 January 2005<br>See Page 13 line 18-Page 45 line 22. | 1-6 |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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|---|---|
| 17 DECEMBER 2007 (17.12.2007) | **17 DECEMBER 2007 (17.12.2007)** |


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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/KR2007/004395**

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